Anatomical and Physiological Studies of the Auditory Pathway in Crickets

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Anatomical and Physiological Studies of the Auditory Pathway in Crickets

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I. INTRODUCTION

Historically, interest in animal behavior dates back to the origin of man and probably stems from his survival instinct, his desire to understand life, and his innate curiosity. The first records of studies on acoustic behavior in insects are found in portions of the Iliad assigned to Homer which were written in the tenth or eleventh century B.C. The first experimental studies on phonotactic behavior in crickets were reported by Regen (1913). He showed classically that females can phonotactically locate a calling male in the absence of other sensory cues. Cricket acoustic behavior has since been used as the basis for a variety of studies.

In most cricket species only the males sing; the sound is produced by specialized structures associated with the anterior wings. A scraper on the edge of one wing rubs against a file on the ventral surface of the other wing as they close over one another (Pierce, 1948; Davis,1968; for a review see Dumortier, 1963a). This sets up a resonation of the harp, thus producing a sound pulse (syllable) with a distinct frequency spectrum (Nocke, 1971). The physical structure of this sound-producing mechanism and the rate at which the wings are repeatedly opened and closed determine the temporal pattern of a song (Walker, 1962; Dumortier, 1963b).

Both males and females possess tympanal structures and auditory organs which are located in the tibial part of the front legs (Burmeister, 1832; Herbig, 1902). Both sexes therefore, are capable of hearing sounds produced by males.

Many species have a repertoire of three song types designed for specific social situations: (1) a calling song for attracting sexually responsive females, (2) a courtship song which induces a phonotactically responsive female to copulate, and (3) an aggressive song used in territorial behavior. These song types vary in temporal pattern and in

frequency spectrum both within a single species and between various cricket species (Zippelius, 1949; Huber, 1955; Alexander, 1960 and 1961; Walker, 1962; Dumortier, 1963c; Weber, 1978).

Species sharing the same habitat have distinctly different calling songs (Alexander, 1967). Experimental evidence for specificity of the calling song in several species of tree crickets has been provided by Walker (1957). Hill et al. (1972) showed that two sympatric species of flying crickets, Teleogryllus commodus and T. oceanicus, could distinguish among simultaneously-presented natural calling songs of the two species.

Later experiments (Hill, 1974) established that positive phonotaxis in T. commodus is sharply tuned to the carrier frequency of the natural calling song. Popov et al. (1975) showed the carrier frequency, ie. the frequency of main sound energy, to be the single most important frequency needed to induce positive phonotaxis in Gryllus bimaculatus and that higher frequency signals induce a negative phonotaxis (see also Popov in Elsner and Popov, 1978). Similar results were reported in the flying cricket T. oceanicus by Moiseff et al. (1978).

In contrast, Zaretsky (1972) established the temporal pattern of the calling song as being the single most important factor governing species specificity in Scapsipedus marginatus. He showed that when using temporal patterns of the natural species-specific calling song, gross changes in the carrier frequency had little influence on phonotaxis.

Purely behavioral experiments led to an interest in the nervous system since phonotaxis is a fairly well-defined response to a specific sensory input. Questions which have been asked by various experimenters are: What does a cricket hear? How does the nervous system process information in the frequency and temporal domain in order to allow a

female to identify a conspecific male? How does a female locate a calling male?

Field crickets have a four-channel input to each tympanal organ (Hill and Boyan, 1976 and 1977; Larsen and Michelsen, 1978; Kleindienst et al., 1980), resulting from an internal tracheal connection between the two tympana and spiracular openings on the prothorax. The tracheal structure has been shown to play a role in determining the effective sound pressure which acts on the tympanal organ (Zhantiev et al., 1975a; Hill and Boyan, 1976 and 1977; Paton et al., 1977; Larsen and Michelsen, 1978; Kleindienst et al., 1980), thus biophysically contributing to directional sensitivity at the level of the tympanal organ.

Within the nervous system itself, auditory receptors have been physiologically studied in some detail in various field crickets (Loftus-Hills et al., 1971; Nocke, 1972; Zhantiev and Tshukanov, 1972a; Eibl, 1976; Zaretsky and Eibl, 1978; Eibl and Huber, 1979; Esch et al., 1980), and in general show that the ear is best tuned to the carrier frequencies of the species-specific songs.

In addition, a wide variety of interneurons have been studied physiologically in an effort to learn how auditory information is processed by the nervous system (Suga, 1963; Zaretsky, 1971; Stout and Huber, 1972; Zhantiev and Tshukanov, 1972b; Popov, 1973; Hill, 1974; Popov et al., 1974; Zhantiev et al., 1975a and b; Rheinlaender et al., 1976; Hill and Boyan, 1976 and 1977; Casaday and Hoy, 1977; Zhantiev and Korsunovskaya, 1977; Zhantiev and Kalinkina, 1977; Elepfandt, 1978; Huber and Stout, 1978; Popov et al., 1978; Popov in Elsner and Popov, 1978; Wiese, 1978; Wohlers and Huber, 1978a and b; Kleindienst et al., 1980; Moiseff and Hoy, 1980; Stout and Huber, 1980).

Cobalt staining techniques (Pitman et al., 1972; Kater and Nicholson, 1973) combined with intensification procedures

(Tyrer and Bell, 1974; Bacon and Altman, 1977) have proved valuable for anatomical studies of the peripheral auditory structures and central projections of auditory afferent fibers (Eibl, 1976 and 1978; Popov et al., 1978; Zaretsky and Eibl, 1978; Eibl and Huber, 1979). Cobalt-filled electrodes (Rehbein et al., 1974) are now being used to extracellularly monitor and simultaneously stain cricket auditory units (Casaday and Hoy, 1977; Popov et al., 1978; Popov in Elsner and Popov, 1978; Wiese, 1978; Esch et al., 1980), allowing correlations to be made between structure and function. Such combined studies should lead to a better understanding of the way the nervous system receives and processes auditory information, ultimately releasing a phonotactic response in the female.

Aims of this study are the following: (1) to more precisely define the cricket "auditory neuropile" in the prothoracic ganglion described by Rehbein (1973), Eibl (1976) and Eibl and Huber (1979), (2) to classify single auditory receptor cells according to their responses and detailed central morphology (Eibl, 1976; Eibl and Huber, 1979; Esch et al., 1980), (3) to present an extensive physiological and morphological characterization of a large segmental auditory interneuron, the omega cell, which has already been studied to some extent in several cricket species (Casaday and Hoy, 1977; Popov et al., 1978; Wiese, 1978; Wohlers and Huber, 1978a and b). Both extra- and intracellular recording techniques are used; cobalt staining methods reveal morphology of nervous structures. The significance of the neurobiological findings are discussed with respect to those of others.

II. MATERIALS AND METHODS

Experimental animals were adult female crickets (Gryllus campestris L.) one to ten weeks after adult molt (Fig. 1), having been caught as late instar nymphs in South Tirol and Kaiserstuhl areas or reared as F1 offspring of those caught. All adults lived in isolated environments.

A. Histological procedures

Morphological studies on the central projections of elements found in the tympanal nerve were initiated by exposing the nerve at the tibial-femoral joint of the foreleg. The nerve was cut and the proximal end placed in a small vaseline "cup" filled with 2% cobalt chloride (Mason, 1973; O'Shea et al., 1974). After sealing off the whole exposed area with vaseline, the animal was placed in a moist chamber maintained at 6°C and left for 20-36 hrs, allowing for the uptake of cobalt ions by cells with processes in the tympanal nerve and projections in the prothoracic ganglion. The ganglion was then removed and treated with 6 drops of ammonium sulphide in 10 ml of Eibl ringer (Eibl, 1976) for 10 minutes. This caused the formation of a dark cobalt sulphide precipitate within cells containing cobalt ions. Preparations were then placed in cricket ringer for 20 min to rinse out excess sulphide ions, and fixed for 2 hrs in alcoholic Bouin. Whole ganglia were subjected to the Timms intensification procedure reported by Bacon and Altman (1977), dehydrated, cleared in methyl salycilate, and mounted in canada balsam for viewing. Some preparations were embedded in soft araldite and sectioned (40 µm thick horizontal, transverse, sagittal) for better resolution of fine details, simultaneously facilitating more precise spatial characterization of processes within the ganglion. Both wholemount and sectioned preparations were photographed using a Leitz Orthomat-W microscope camera system mounted on a Leitz Orthoplan microscope. Reconstructions were made

Fig. 1 The frontal portion of a male Gryllus campestris showing the orientation of the large tympanum (arrow) on the foreleg.

using a Wild M20 microscope equipped with a drawing tube. Afferent fibers associated with single sensory hairs and campaniform sensillae on the tibia were selectively stained by placing a drop of cobalt on the cuticle directly over sensory hairs or campaniform sensillae and allowing the sensory cells to take up cobalt ions.

B. Physiological procedures

1. Animal preparation

Animals to be used for electrophysiology were pinned ventral side up on a plexiglass table following the removal of wings and antennae. The forelegs were fixed in a position similar to an inverted standing posture with the large tympana (Fig. 2) about 4 mm above the surface of the preparation table. The prothoracic ganglion was exposed, care being taken to preserve the tracheal system. The animal was then placed in the middle of a recording table in such a way that the large tympana faced two opposing external loudspeakers (Fig. 3) located within the horizontal plane of the animal and 60 cm from each ipsilateral tympanum. A faraday cage lined with sound-absorbing glass wool enclosed the recording table. A silver spoon which also served as the indifferent electrode was placed under the ganglion for support (Fig. 4), but the ganglion was not lifted from its position in the body. In experiments where morphology was studied, microelectrodes filled with 1 M cobalt nitrate (Kien, 1976) were used (resistance 60-100 MOhms). Physiological data from morphologically identified units was collected using electrodes filled with 3 M potassium acetate (resistance 40-70 MOhms). The ganglion sheath itself was not treated; Fieldens saline (Fielden, 1960) was used to keep the preparation moist. Being slightly hypotonic, it tended to stretch the sheath enough to allow penetration of the microelectrode tip without breakage. The electrode was advanced at a right angle to the ganglion surface with a

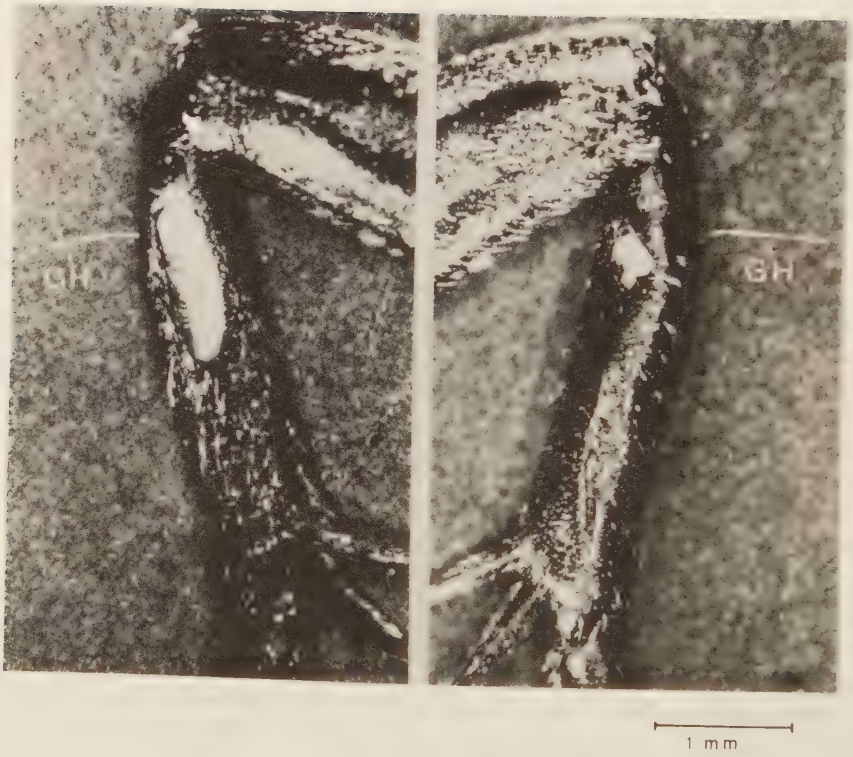


Fig. 2 The foreleg tibia from a female G. campestris.

Left: The large tympanum located on the posterior side of the tibia and the guard hair (GH), a sensory hair on the outside of the tibia.

Right: The small tympanum located on the anterior side of the tibia.

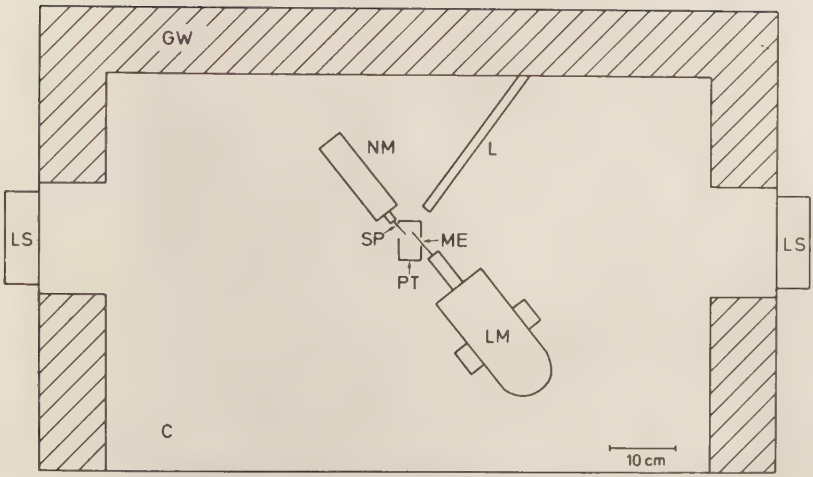


Fig. 3 Diagram of the open-field stimulus system used for acoustic stimulation when recording from cricket auditory afferent fibers and interneurons. Labels: C, vibration-dampened cage with glass wool (GW) for absorption of sound signals; L, light guide; LM, Leitz micromanipulator holding the microelectrode (ME); LS, loudspeakers; NM, Narishige micromanipulator holding the silver spoon (SP) which supports the prothoracic ganglion; PT, preparation table. (Modified from Wohlers and Huber, 1978)

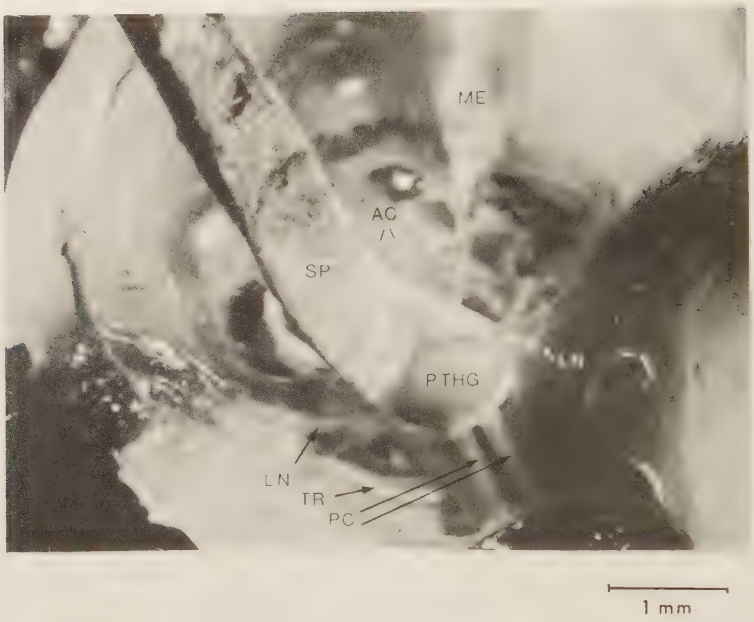


Fig. 4 Exposed prothoracic ganglion (PTHG) supported by a silver spoon (SP, also serving as the indifferent electrode). Other labels: AC, anterior connectives; LN, leg nerves; ME, glass capillary microelectrode (recording electrode); PC, posterior connectives; TR, trachea connecting the two tympana internally.

Leitz micromanipulator. Touching of the sheath was noted by a sudden increase of electrode resistance. The electrode was then advanced only 4-5 µm at a time, the case of the micromanipulator was tapped lightly several times with a finger, and the procedure was then repeated. Sometimes it took 5 minutes to penetrate the sheath, but once inside the ganglion the electrode resistance was the same as, or slightly higher than it had been outside the ganglion. The same procedure was used for locating and penetrating cells. For studies of auditory afferent fibers the electrode was positioned where the leg nerve enters the prothoracic ganglion. Interneurons were penetrated from auditory neuropilar regions of the ganglion.

2. Open-field stimulation

Temporal characteristics of the acoustic stimuli were generated by a W-P Instruments Interval Generator Model 830. The syllable envelopes were shaped and timed with a Wavetek Model 112. Another Wavetek (Model 136) with VCG input was used to generate the carrier frequency. Acoustic signals were presented to the animal through a Bruel and Kjaer Type 2706 Power Amplifier driving Isophon KKS 10-11 speakers. To reduce the problem of standing waves in the system, an electronic device designed by Dr. U. Koch was used. With this device as an integral part of the sound-producing circuit the intensity (in dB SPL) of a constant tone at all relevant frequencies of sound was calibrated with a Bruel and Kjaer one-half inch Condensor microphone Type 4133 connected to a Bruel and Kjaer Type 2607 Measuring Amplifier. The microphone was placed at the position which would later be occupied by the ear ipsilateral to the speaker being tested. The intensity at each frequency was adjusted to the desired level with potentiometers built into the sound-producing circuit. In the open-field situation the intensity of sound reaching the contralateral side could not be controlled. Temporal parameters of artificial calling songs resembled those of the calling song

of G. campestris (Huber, 1977; Weber, 1978). A standard chirp consisted of four syllables with sinusoidal envelope, equal amplitude, and 20 ms duration, each being separated by 15 ms pauses. Such a chirp was repeated every 400 ms. In most cases 11-25 chirps were presented in a series. Variations of the standard chirp included pure tones of various durations and envelopes. Intensity, measured in dB SPL (0 dB = 2 x 10^{-5} N/m^2), always refers to peak intensity independent of the shape of the envelope used.

3. Closed-field stimulation

In order to acoustically isolate each tympanum externally, special "legphones" were developed whereby closed-field stimulation of each ear was made possible. Here animals were mounted ventral side up on a lucite sledge which could be adjusted along a dovetail guide supporting small sound cavities (Fig. 5). These cavities were made of plexiglass tubes having an inside diameter of 6 mm and a length of 3 mm (Fig. 6). A condensor microphone (Bruel and Kjaer 1/4 inch, Type 4135) served as the sound source, the diaphragm of which formed the back wall of the cavity. Two grooves on the side allowed convenient insertion of the cricket forelegs. The front wall was formed by another Bruel and Kjaer 1/4 inch microphone, for calibrating and measuring sound intensities, or by a brass cap. Because of the small inside dimensions the lowest resonant frequency within the cavities occurred at 40 kHz, well above the range (2-20 kHz) used in this study (see Kleindienst et al., 1980 for a full description of the legphones and for theoretical considerations). Two such cavities were calibrated to the same output within 0.2 dB in the frequency range of main interest (2-20 kHz), one for each ear. Figure 7 diagrammatically shows the entire stimulus generating system used to drive the legphones. Both channels were fed by the same sine generator. The sine voltages in each channel were transformed to sine wave pulses with two identical modulators and an envelope

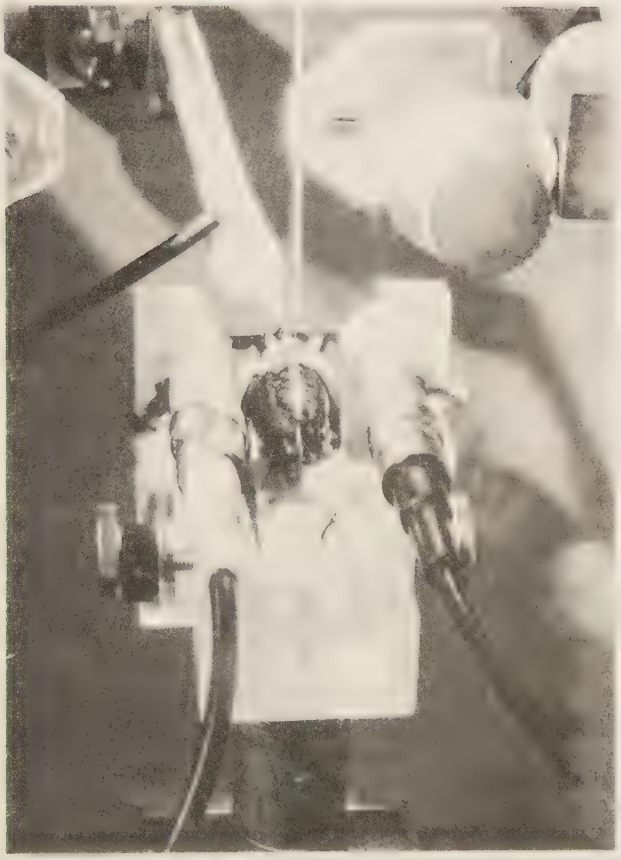


Fig. 5 The closed-field stimulus system used to acoustically isolate the two large tympana externally.

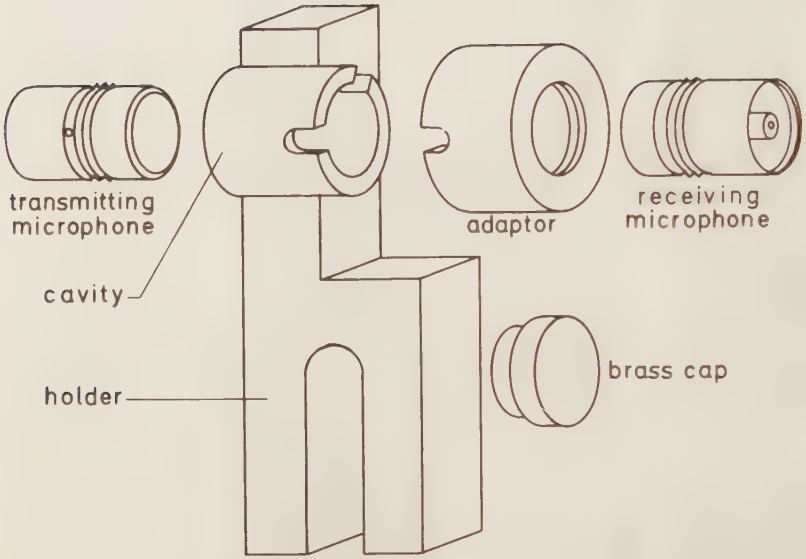


Fig. 6 Diagram showing the detailed construction of the sound cavities into which the cricket forelegs are placed for closed-field stimulation. The actual inside dimensions of the cavities are: Length- 3mm, diameter- 6mm. (After Kleindienst et al., 1980)

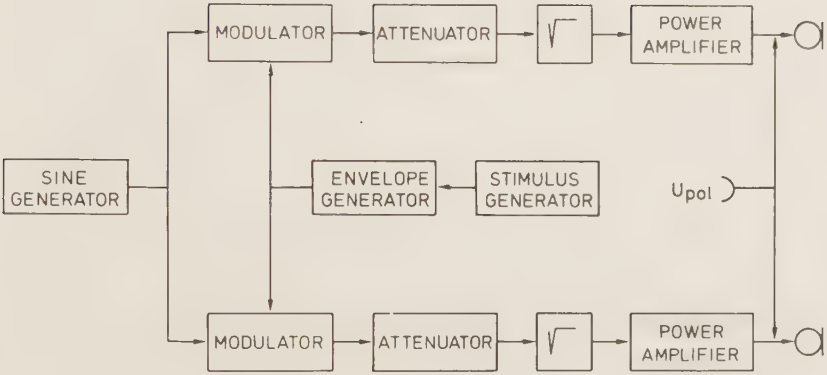


Fig. 7 Schematic diagram of the stimulus-generating system used in combination with the closed-field stimulus system.

generator. The envelope generator signal thus controlled pulse repetition rate, pulse duration, and ramp slope in both channels. Step attenuators were used to set the gross sound pressure level in the cavities in 5 dB steps. Pulse distortions at the sound-producing diaphragm were eliminated by pre-distorting the generated sound (opposite polarity to that produced at the diaphragm) using a square-rooter (see Kleindienst et al., 1980 for a detailed explanation). This distorted signal was then amplified with Bruel and Kjaer Type 2706 Power Amplifiers driving the sound-producing microphones. In all experiments involving the legphones the prothoracic spiracles were waxed over to eliminate them as acoustic inputs to the tympanal organs, simultaneously creating reproducible experimental conditions. When the cricket forelegs were properly fixed in the sound cavities, intensity differences between inside and outside environment were greater than 60 dB SPL.

4. Data collection and analysis

Nervous activity, monitored through headphones, was amplified with a Getting Preamplifier and stored as a DC signal on a Racal Store 4D tape recorder. A DEC PDP11-40 computer equipped with graphics was used for data analysis. When staining units, electrodes filled with 1 M cobalt nitrate were used and cobalt ions were simply allowed to diffuse into the cell for 30 min to 2 hrs. The stained tissue was then processed as described earlier for fills of the tympanal nerve.

5. Characterization of cricket calling song parameters

The building block of the calling song is the syllable produced when a male closes one wing over the other. It has a characteristic envelope and species-specific sound frequency spectrum, the frequency with the highest level of energy being termed the carrier frequency. The calling song of G. campestris is typically composed of chirps

containing four syllables which are separated by pauses. Figure 8 summarizes the terminology used to characterize the temporal parameters.

III. RESULTS

A. Central projections of elements in the tympanal nerve

The tympanal nerve of crickets is a sensory nerve receiving axons from four different sensory structures: the tympanal organ, the subgenual organ, tibial sensory hairs, and a group of campaniform sensillae located on the outside of the tibia just proximal to the cuticular crease (Fig. 9; see also Eibl, 1976 and 1978). Cobalt fills of the whole tympanal nerve reveal a compact bundle of sensory fibers which travel the length of the tympanal nerve, joining the leg nerve within the coxa. This bundle remains a unit, travelling to within a few hundred microns of the prothoracic ganglion before separating into two bundles (Fig. 10a), a more anterior and a more posterior one. They enter the prothoracic ganglion as distinctly separate bundles (Fig. 10b). In general, both bundles course ventrally within the leg nerve (Fig. 10c) although a few individual fibers may be found scattered throughout the nerve.

Several types of projections are found in the prothoracic ganglion itself (Figs. 11 and 12). The most conspicuous projections form a crescent-shaped structure, within intermediate neuropile (Fig. 11), near the midline of the ganglion. It clearly is composed of two types of sensory fibers as illustrated in Figure 11b. In addition, a more ventral neuropile can be seen along with a few large fibers projecting more dorsally with respect to the intermediate neuropile (Fig. 12).

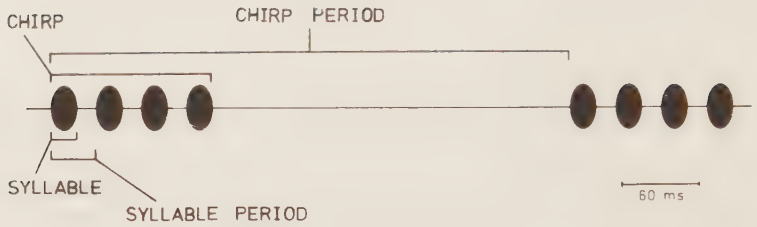


Fig. 8 Terminology used in defining the temporal parameters of the calling song of G. campestris, shown schematically.

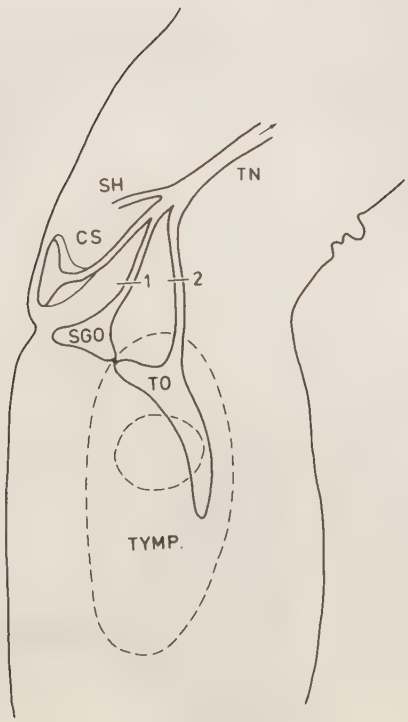


Fig. 9 Schematic drawing showing the peripheral innervation pattern of the cricket tympanal nerve (TN) within the proximal tibia (frontal view of left tibia). Labels: CS, campaniform sensillae; SGO, subgenual organ; SH, branch to tibial sensory hairs; TO, tympanal organ; TYMP, large tympanum. 1 and 2 refer to the position from which ultra-thin EM sections of the subgenual (1) and tympanal (2) nerve branches, shown in Fig. 17, were taken. Arrow indicates proximal. (Sketched from Eibl, 1978)

Fig. 10 Cobalt-filled fibers from the tympanal nerve.

a. Wholemount showing the tympanal-nerve fibers (TF) within the proximal leg nerve (LN) as they separate into two bundles, an anterior one (AB) and a posterior one (PB) a few hundred microns distal to the prothoracic ganglion (PTHG). Arrow indicates anterior.

b. View of the anterior (AB) and posterior (PB) bundles of tympanal-nerve fibers as they enter the prothoracic ganglion (PTHG). Arrow indicates anterior.

c. View of a thick section taken through the leg nerve near its entrance to the prothoracic ganglion revealing the position of the anterior (AB) and posterior (PB) bundles within the leg nerve. Horizontal arrow indicates posterior, vertical arrow indicates dorsal.

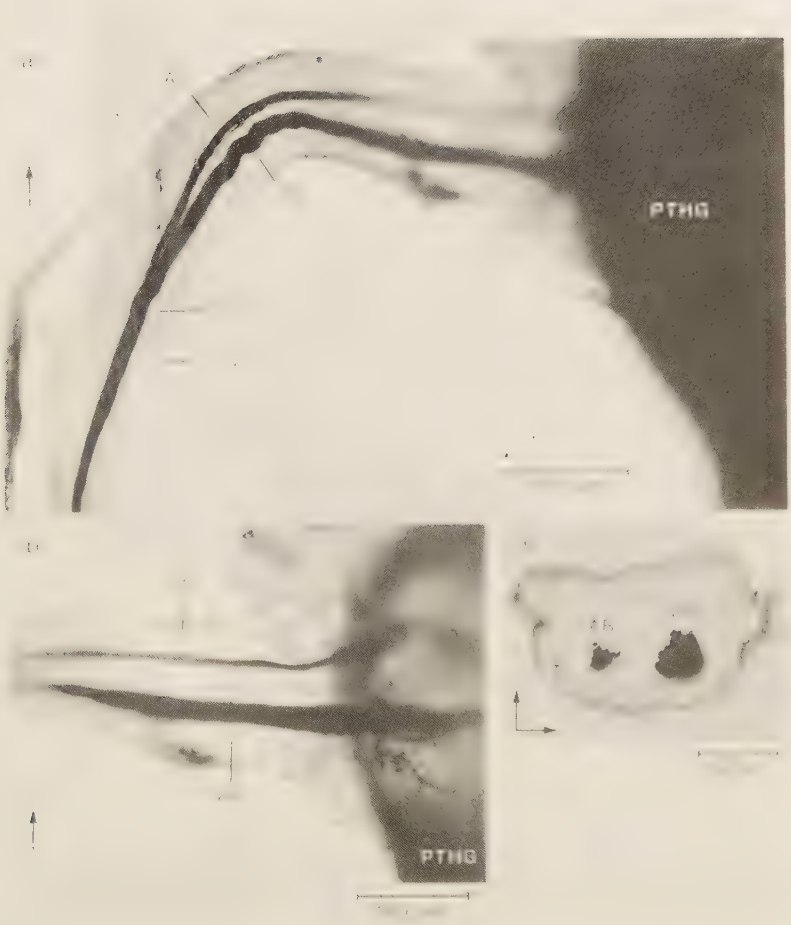
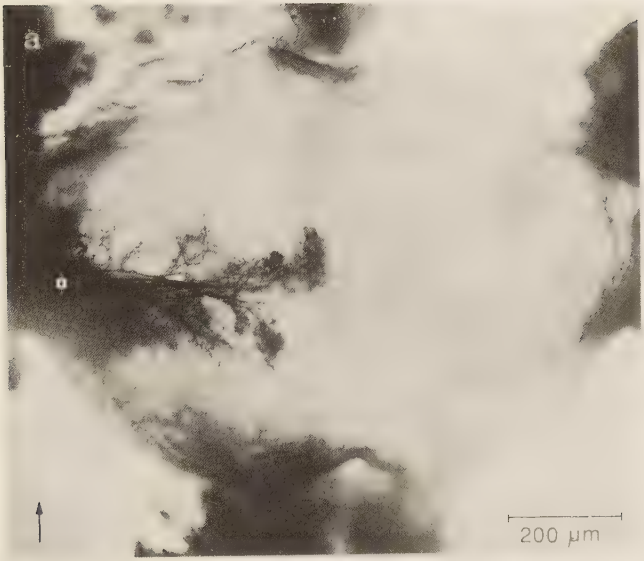

PTHG
PTHG

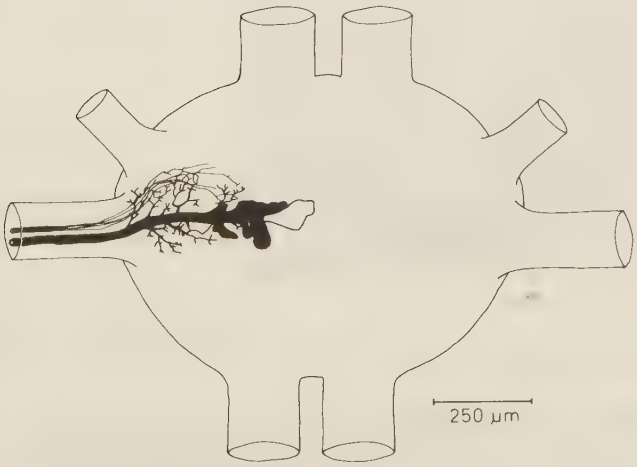
Fig. 11 Central projections of fibers with axons in the tympanal nerve, visualized through cobalt-filling of the whole tympanal nerve. Arrow indicates anterior.

a. Horizontal photograph of a wholemount.

b. Horizontal reconstruction showing various fiber types which can be morphologically classified according to their central projection pattern.



b



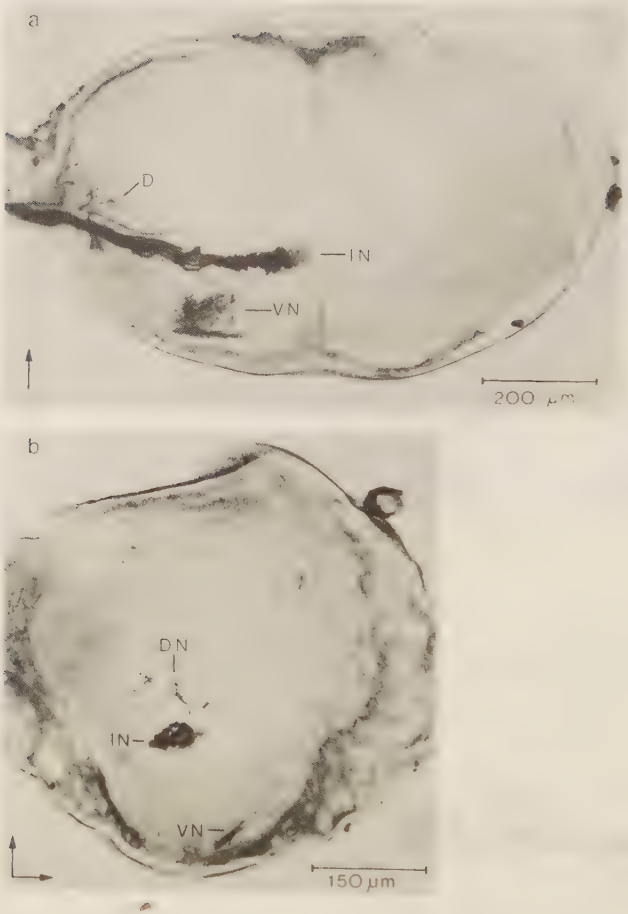


Fig. 12 Central projections of tympanal-nerve fibers photographed from thick sections.

a. Transverse section through the bundle of fibers contributing to intermediate neuropile (IN) and coursing to the midline of the prothoracic ganglion. A projection of fibers to ventral neuropile (VN) is also seen along with a few fibers near the lateral edge of the ganglion which project more dorsally (D). Arrow indicates dorsal.

b. Sagittal section through the middle of the left lateral hemisphere of the prothoracic ganglion to demonstrate the relative positions of fibers projecting to ventral (VN), intermediate (IN) and more dorsal (DN) neuropile. Horizontal arrow indicates anterior, vertical arrow indicates dorsal.

The ventral projections all arise from the more anterior of the two fiber bundles entering the prothoracic ganglion (Figs. 10, 11b, and 13). Individual fibers are relatively small (ca. 1 µm in diameter) and can be seen to branch immediately upon entering the ganglion while also turning anteriorly and ventrally to branch freely as they course toward the midline (Fig. 13a and b). All projections remain ipsilateral to the side of entry however. Selective fills of an identified sensory hair cell, the guard hair (Fig. 2) studied in detail by Neumann (1975), reveal an identical pathway of projection (Fig. 13c). Since it is known that sensory fibers from many tibial hairs course in the tympanal nerve (Eibl, 1976 and 1978), this whole bundle is assumed to arise from such sensory fibers.

Cells innervating the campaniform sensillae were filled selectively by placing a drop of cobalt on the cuticle directly over the sensillae. Since the cuticle in this region is softer than surrounding cuticle, cobalt more readily penetrates it and is taken up by the associated sensory cells. The axons are seen to course centrally along the more posterior bundle of fibers entering the prothoracic ganglion (Fig. 14a, see also Fig. 11b). They appear to be large in size (larger than 3 µm in diameter) and branch extensively upon entering the ganglion, arborizing both anteriorly and posteriorly. The main branches course toward the midline, but remain strictly ipsilateral (Fig. 14). Most branches are found within intermediate and dorsal neuropile; they are especially conspicuous at the lateral edge of the ganglion (Fig. 14b).

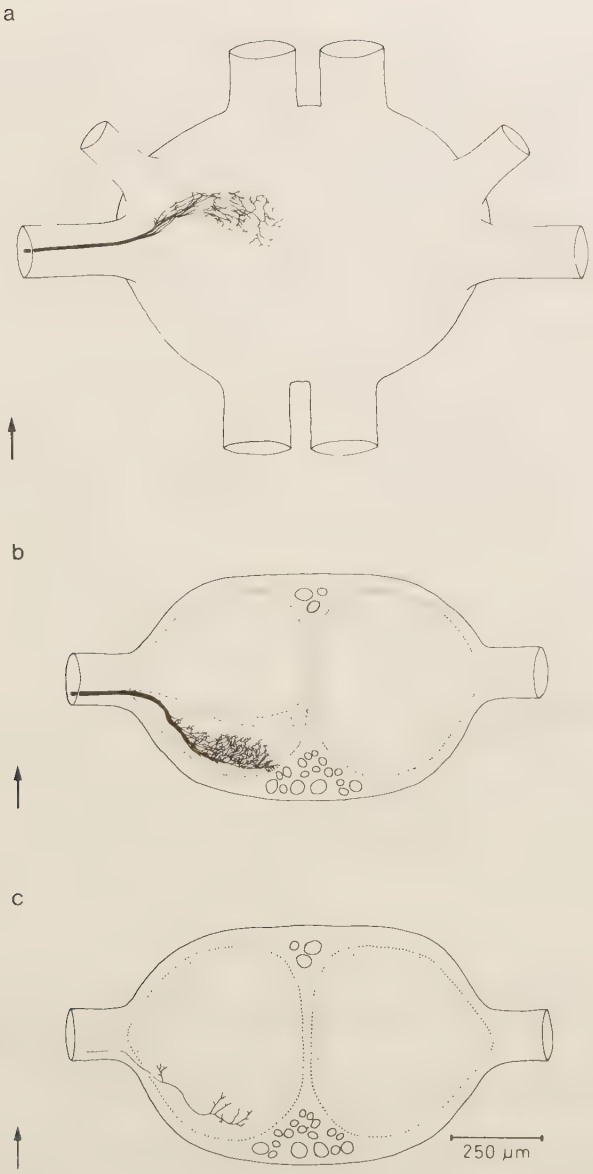
Because of the close proximity between the tympanal and subgenual organs it was not possible to selectively stain sensory cells associated with each structure using the cobalt methods described here. However, the crescent-shaped neuropilar structure described by Eibl (1976) was consistently revealed in fills of the whole tympanal nerve (Fig. 15); it arises from the posterior bundle of fibers

Fig. 13 Reconstructions of the central projections of fibers from the tympanal nerve which enter the prothoracic ganglion via the anterior bundle shown in Fig. 10.

a. Drawing in the horizontal plane. Arrow indicates anterior.

b. Drawing in the transverse plane. The dotted line represents projections of fibers to intermediate neuropile. Arrow indicates dorsal.

c. Central projections of a single tibial sensory hair, the guard hair, showing its arborization within ventral neuropile. Arrow indicates dorsal.


a
b
c
250 µm

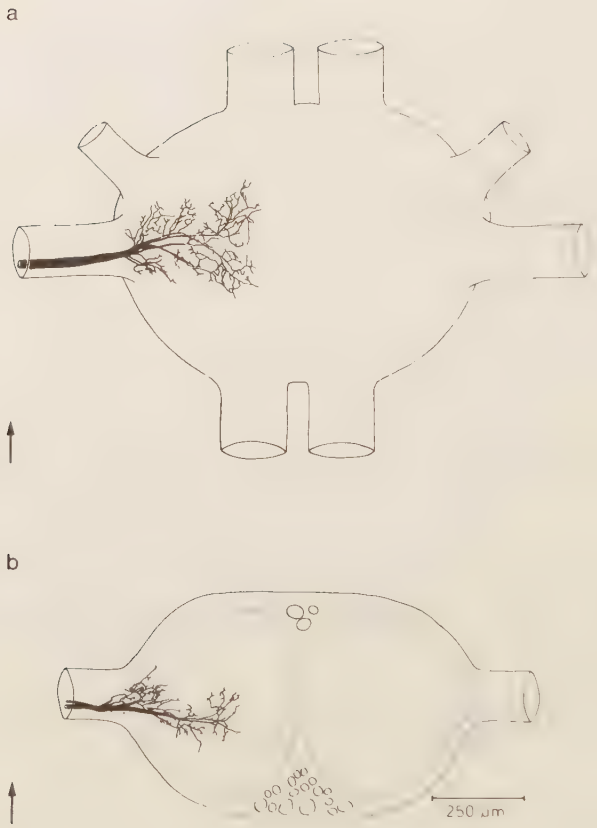


Fig. 14 Reconstructions of the central projections of selectively-stained campaniform sensillae.

a. Horizontal view. The fibers can be seen to course in the posterior bundle entering the prothoracic ganglion from the tympanal nerve. Arrow indicates anterior.

b. Transverse view showing the relationship of the campaniform sensillae projections to those contributing only to the intermediate neuropile (dotted line). Arrow indicates dorsal.

entering the prothoracic ganglion. Since this structure is not associated with sensory hairs or campaniform sensillae (see Figs. 13 and 14), it presumably outlines tympanal and subgenual receptor endings. Two fiber types can be classified according to their relative size and projection pattern, and are seen as two distinct bundles (Fig. 15). One bundle fills more lightly than the other. Fibers within this bundle appear to be small in size and densely packed, running toward the middle of the ganglion where they terminate in a more or less ball shape. They are the most medially-projecting fibers of all those from the tympanal nerve and appear to be an extension of the anterior part of the crescent-shaped structure. The more darkly-staining fibers are relatively large (3-4 μm in diameter) and more diffuse; there appear to be 10-15 such fibers. They too run toward the midline upon entering the ganglion, branching laterally and terminating medially by bifurcating anteriorly and posteriorly, lateral to the more medial projections of the bundle which stains more lightly.

Both bundles contributing to the crescent-shaped neuropile terminate within intermediate neuropile (Fig. 16). Likewise, the two bundles run in the same tract such that no dorsal-ventral separation can be seen, ie. the bundle of lightly-staining fibers courses through the middle of the neuropile formed by the larger, more darkly-staining fibers (Fig. 16c and d).

B. Electron-microscope studies of the subgenual and tympanal nerves

Ultra-thin transverse EM sections of the tympanal nerve branches innervating the subgenual (Fig. 17a) and tympanal organs (Fig. 17b) show the distribution of sensory fibers from each organ (see Fig. 9 for a description of the location at which the sections were taken). Approximately 20 axons ranging from 2 to 4 μm in diameter are found in the subgenual branch. The auditory branch contains on the

Fig. 15 The organization of the crescent-shaped intermediate neuropile, photographed horizontally and viewed from the ventral surface. Arrow indicates anterior.

a. Photograph of the whole prothoracic ganglion. The fibers projecting from the right toward the midline are composed of two types, classified by their relative size, their cobalt-uptake, and their central structure. Though both tympanal nerves were filled in this preparation the larger fibers contributing to the more lateral portions of the crescent-shaped neuropile (darker fibers on the right) failed to stain on the left.

b. Enlarged photograph of the crescent-shaped neuropile showing more distinctly the two fiber types.

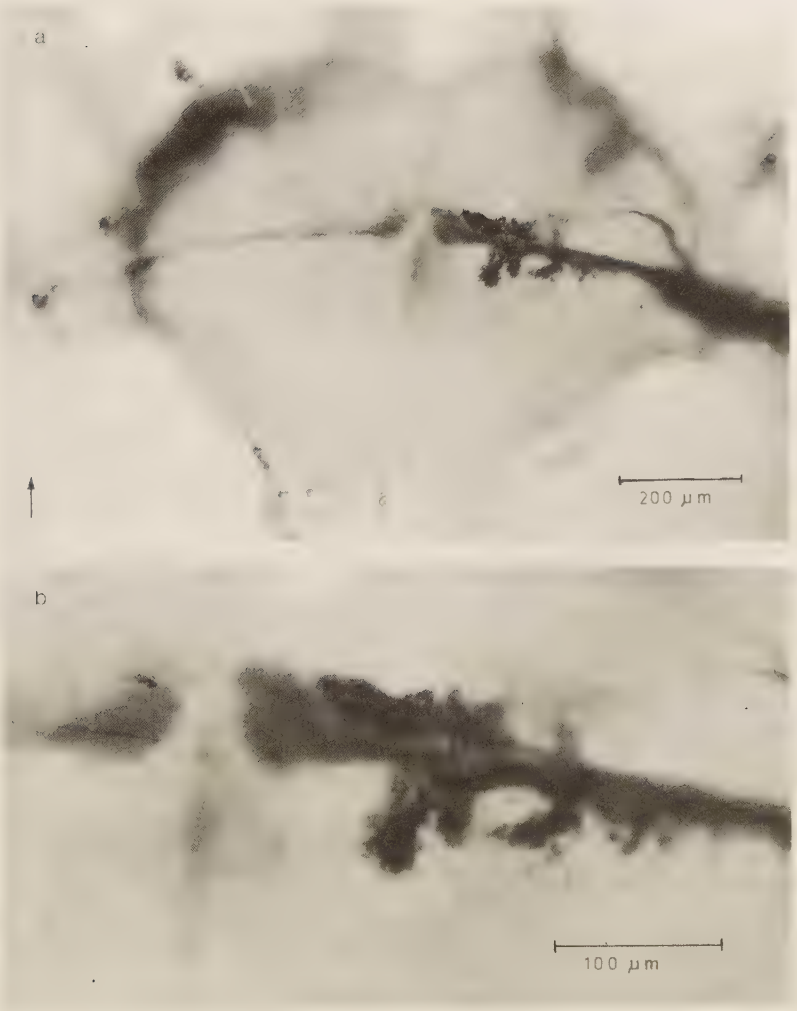

a
200 µm
b
100 µm

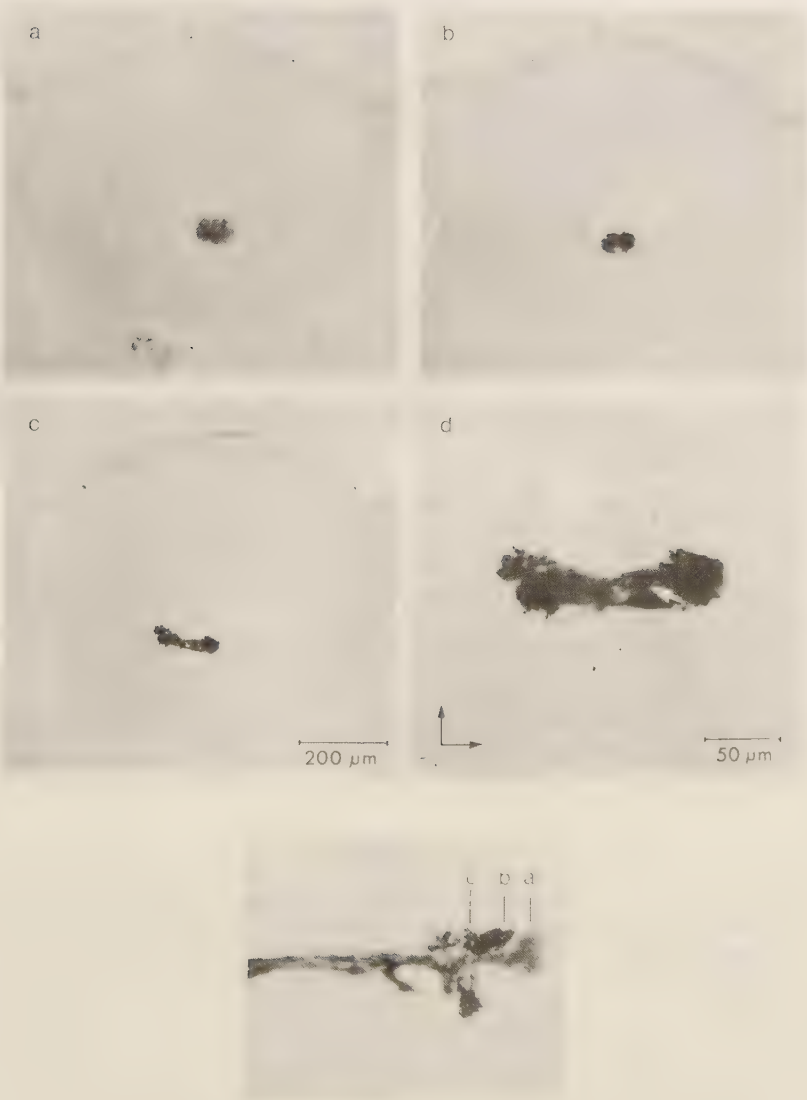
Fig. 16 Photographs, from sagittal sections, of a cobalt-filled preparation. Sections were taken at the positions indicated in the photo (bottom). The structure of the crescent-shaped intermediate neuropile is shown in detail. Horizontal arrow indicates anterior, vertical arrow indicates dorsal.

a. Projections of the most medially-projecting bundle of fibers which typically stain rather lightly with cobalt.

b. Section taken from the medial edge of the more darkly-staining fibers to show the lack of a dorsal-ventral separation of fiber types.

c. Section taken from the middle of the more darkly-staining fibers. They are seen to project much more anteriorly and posteriorly, contributing to the lateral portions of the crescent-shaped intermediate neuropile.

d. Enlarged photograph of the section shown in (c). The most medially-projecting bundle of lightly-staining fibers (arrow) passes directly through the arborizations of more darkly-staining fibers.


a
b
c
d
200 µm
50 µm
c
b
a

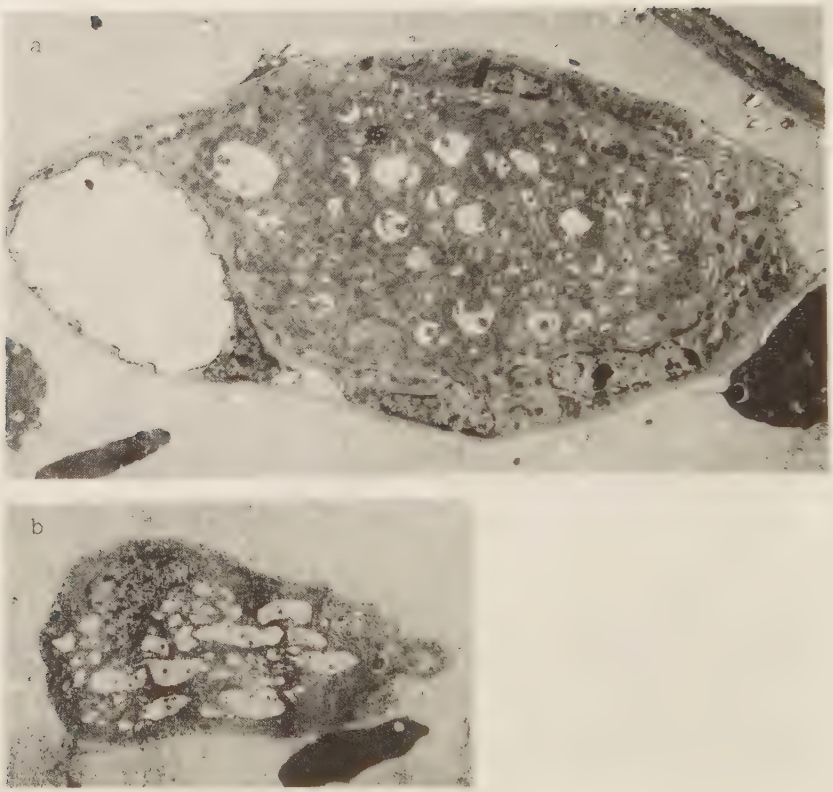
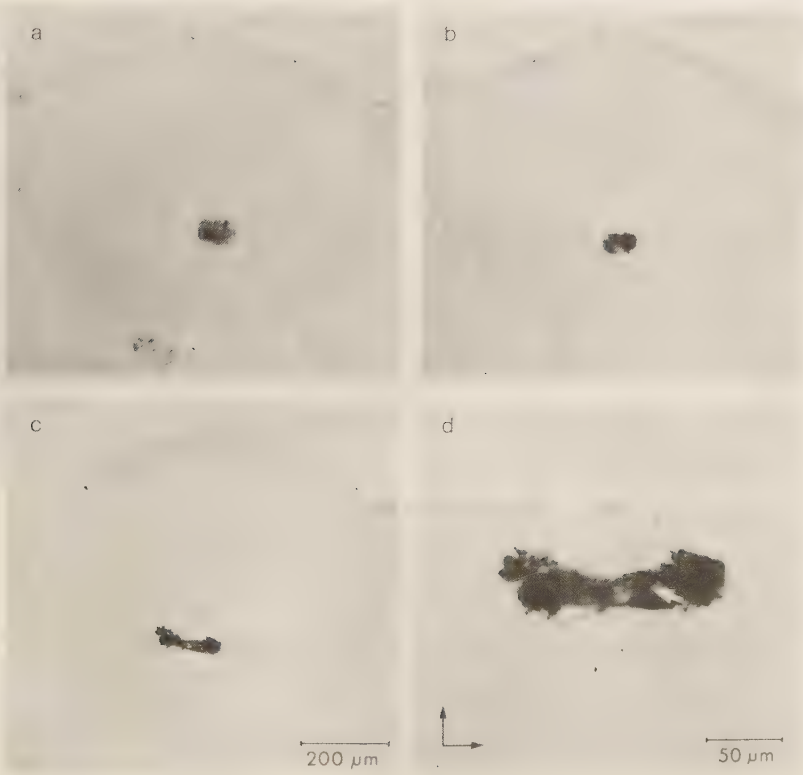
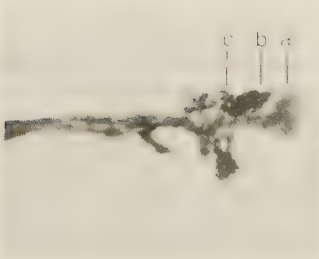


Fig. 17 Photomicrographs of ultra-thin EM sections taken through the subgenual (a) and tympanal (b) branches of the tympanal nerve. Magnification: 4000x. See Fig. 9 for a description of the position at which the sections were taken.


a
b
c
d
200 µm
50 µm


c
b
a

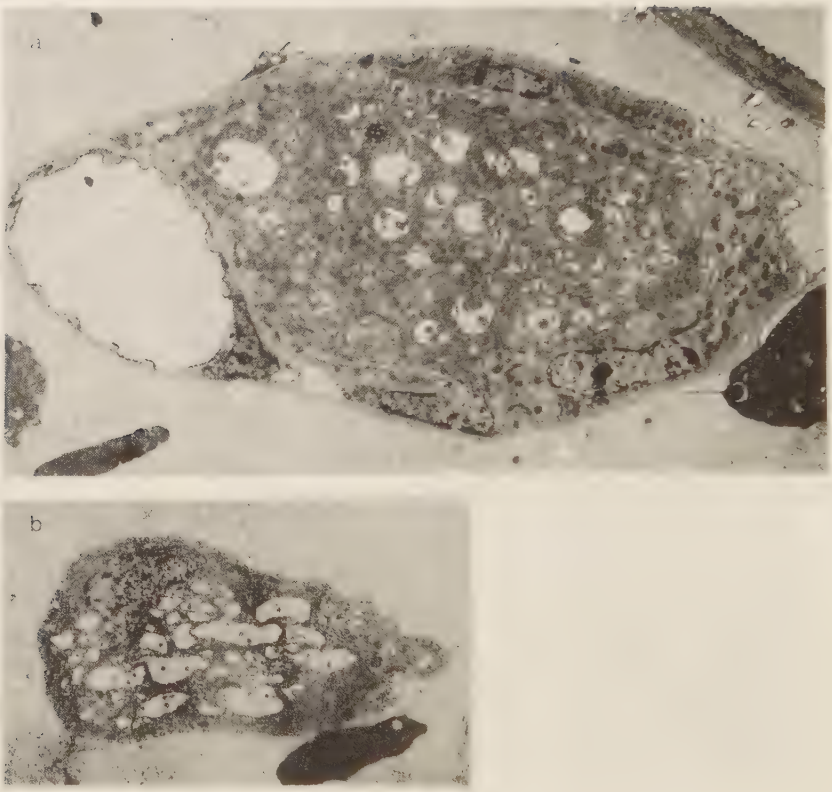


Fig. 17 Photomicrographs of ultra-thin EM sections taken through the subgenual (a) and tympanal (b) branches of the tympanal nerve. Magnification: 4000x. See Fig. 9 for a description of the position at which the sections were taken.

order of 60-70 small axons (diameter 0.5 to 1 μm) and about 12 larger ones (diameter 2 to 4 μm).

C. Summary of the fiber types associated with the tympanal nerve

Figure 18 summarizes the results obtained from cobalt fills of both tympanal nerves in horizontal (a), transverse (b), and sagittal (c) views. Projections of all four sensory types can be clearly seen and, from information obtained in selective studies, can be classified according to type and function. In summary, the most anterior bundle of tympanal nerve fibers entering the prothoracic ganglion comes from tibial sensory hair cells which project into ventral neuropile. All other fibers course through the posterior branch where the largest fibers, upon entering the ganglion, branch diffusely both anteriorly and posteriorly to intermediate and dorsal regions of neuropile. The relatively large fibers contributing to the lateral parts of the crescent-shaped intermediate neuropile may largely represent the subgenual organ. The densely-packed and most medially-projecting fibers probably outline a major portion of the auditory neuropile.

D. Physiology and central projections of single auditory receptors

Single auditory receptors were studied under open-field stimulus conditions (see Fig. 3) using extracellular microelectrode recording and staining techniques. The electrode was positioned above the leg nerve near the junction to the prothoracic ganglion (Fig. 19a) and then carefully advanced through the connective tissue layers surrounding the nerve. As the electrode tip approached the posterior nerve bundle containing auditory fibers, nervous activity synchronized to patterned acoustic stimulation could be observed (Fig. 19b). The tip was then carefully advanced until a single auditory

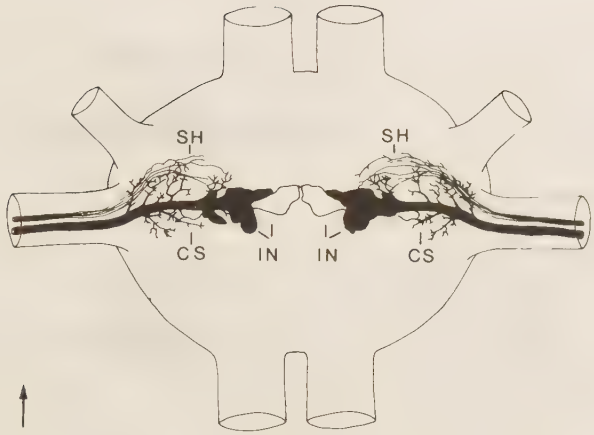
Fig. 18 Reconstructions of the central projections of all cobalt-filled fiber types from the tympanal nerves (both sides). Labels: SH, ventral projections of sensory hairs; CS, intermediate and dorsal projections of campaniform sensillae; IN, crescent-shaped intermediate neuropile composed of fibers from the subgenual and tympanal organs.

a. Drawing in the horizontal plane. Arrow indicates anterior.

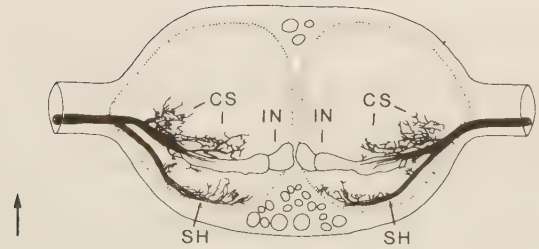
b. Drawing in the transverse plane. Arrow indicates dorsal.

c. Drawing in the sagittal plane. Horizontal arrow indicates posterior, vertical arrow indicates dorsal.

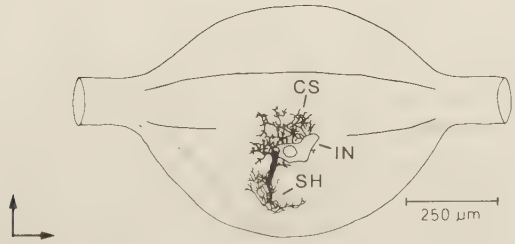
a



b



c



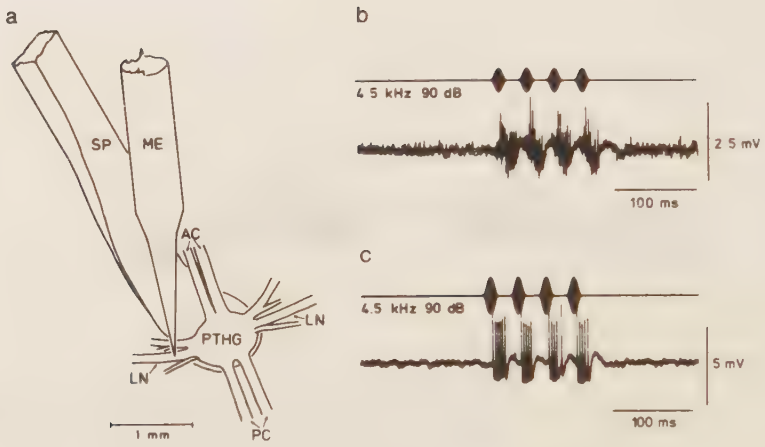


Fig. 19 Microelectrode recordings from afferent fibers.

a. Sketch of the recording situation to show the position at which the electrode was inserted. Labels as in Fig. 4.

b. The summed response of auditory afferent fibers (lower trace) to an artificially-produced calling song (upper trace).

c. Responses of a single auditory afferent fiber (lower trace), best-tuned to 4-5 kHz signals representing the calling song (upper trace).

receptor was isolated (Fig. 19c).

Recordings from many different animals revealed ultimately four receptor types, classified according to their frequency responses and, in part, by their central projections. Often extracellular fills of preparations showing single receptor responses nevertheless resulted in the filling of several fibers.

The stimulus system used was calibrated to deliver signals in the frequency range of 2-20 kHz. In this range one receptor type (type 1) responds best to 2 kHz (probably even lower frequencies), showing a threshold response of 73 dB SPL at 2 kHz (Fig. 20, upper left). The intensity required for a threshold response increases with an increase in sound frequency until at 10 kHz no response is elicited even at 100 dB SPL. At above-threshold stimulation, the receptor accurately codes the temporal parameters of an artificially-produced calling song (Fig. 20, lower left). Using extracellular 1 M cobalt nitrate-filled electrodes, the morphology of this receptor type was studied; the central projections are shown in Figure 20 (middle). Upon entering the ganglion the relatively large axon arborizes diffusely, sending off both anterior and posterior branches as it courses toward the middle of the ganglion. Projections are ipsilateral and appear to terminate in intermediate and dorsal neuropilar regions.

A second type responds to acoustic stimuli from 2-16 kHz (Fig. 21, upper left). It is, however, best tuned to 4.5 kHz which is the frequency of main sound energy in the calling song of G. campestris, and is also adept in coding the temporal parameters of the calling song (Fig. 21, lower left). Cobalt-fills from such cell types always reveal the central projections of several fibers (Fig. 21, middle) even though physiological data shows the response of a single unit (Fig. 21, lower left). None of the filled fibers show arborization at the edge of the ganglion; they

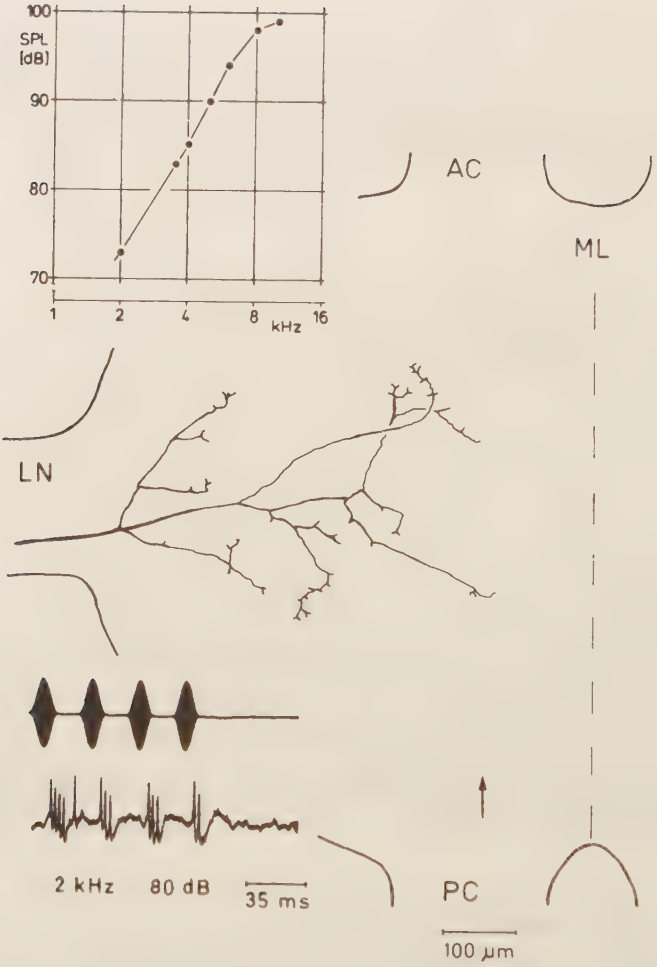


Fig. 20 Central arborizations (horizontal view) of an afferent fiber (middle) best-tuned to 2 kHz or less, as shown by its auditory threshold curve (upper left inset). Its response to the temporal parameters of an artificial calling song presented at 2 kHz is shown (lower left inset). Labels: AC, anterior connective; LN, leg nerve; ML, midline of the ganglion; PC, posterior connective. Arrow indicates anterior.

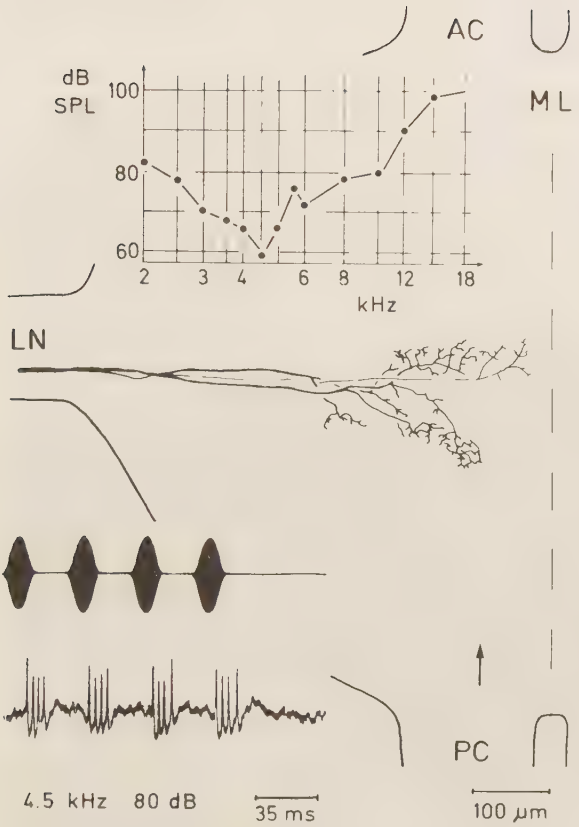


Fig. 21 Reconstruction of afferent fibers (middle, viewed horizontally) stained when recording from a single fiber best-tuned to 4-5 kHz (upper left inset, auditory threshold curve). The patterned response of the single fiber to the temporal parameters of an artificial calling song is seen in the lower left inset. Labels as in Fig. 20. Arrow indicates anterior. (After Esch et al., 1980)

all course toward the midline where they give off short anterior and posterior branches ending in dense arborization. The fibers remain ipsilateral and terminate within intermediate neuropile.

The third receptor type exhibits two sensitivity maxima, 4.5 and 11 kHz (Fig. 22, upper left). Temporal parameters of the calling song are well-copied (Fig. 22, lower left). Cobalt-fills from this cell type also reveal several fibers (Fig. 22, middle); branching is diffuse along the length of the axons from near the lateral edge of the ganglion to the midline. The branches run both anteriorly and posteriorly, are very short, and terminate in intermediate neuropile. All endings are ipsilateral.

Another (fourth type) was recorded only once. Physiologically it shows a sensitivity tuned to higher frequencies, with a best response at 17 kHz (Fig. 23, upper left). Several fibers (Fig. 23), with projections similar to those found in fills of both type 1 and type 2 receptors (see Figs. 20 and 21), were filled, rendering it impossible to characterize this receptor type morphologically.

In Figure 24 the individual auditory receptor types characterized by extracellular recording and staining techniques (see Figs. 20-23) are compared with the crescent-shaped neuropile consisting of fibers from the tympanal and subgenual organs. The Type 1 (2 kHz) receptors closely resemble the campaniform sensillae shown in Figures 14 and 18; they do not project to the crescent-shaped neuropile known to contain auditory receptors (Fig. 24a). Fills made while recording from receptors best tuned to 4.5 kHz (Type 2) reveal the two cell types contributing to the crescent-shaped neuropile (Fig. 24b, see also Fig. 15). Projections of Type 3 receptors, those with sensitivity maxima at 4.5 and 11 kHz, are also contained within the crescent-shaped neuropile (Fig. 24c). Although the fourth type (17 kHz) could not be visualized morphologically, the

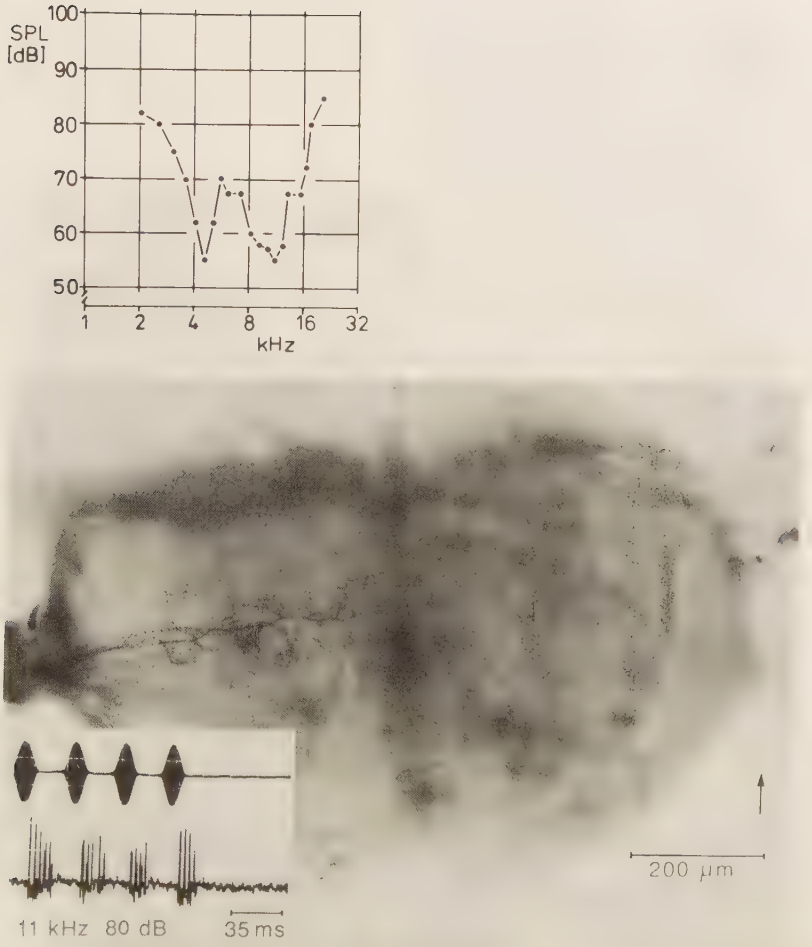


Fig. 22 Horizontal view, from a wholemount, of afferent fibers (middle) stained when recording from a unit with two sensitivity maxima, 4.5 and 11 kHz (seen in the auditory threshold curve, upper left). The patterned response to an artificial calling song, presented at 11 kHz, is shown at the lower left. The presence of several spike amplitudes suggests a multi-unit recording. Arrow indicates anterior.

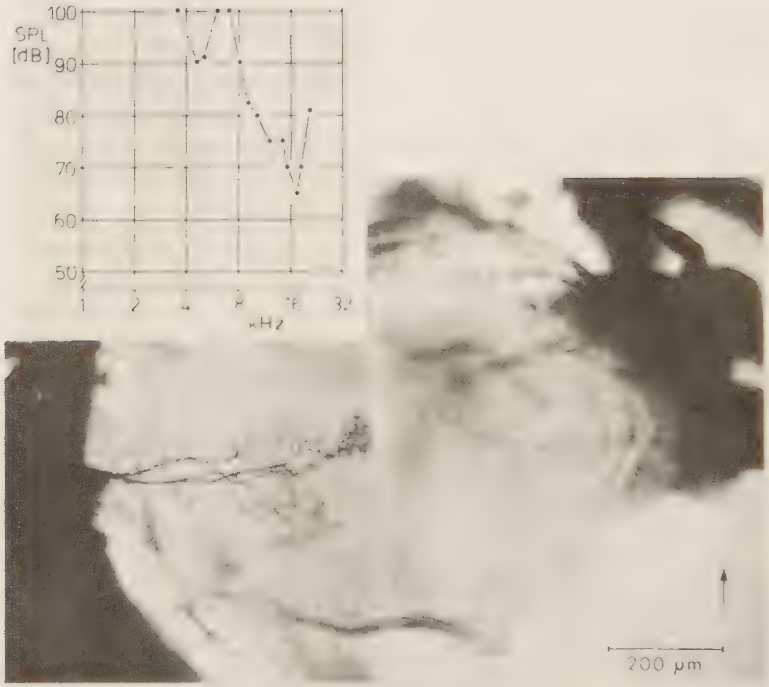


Fig. 23 Horizontal view (wholemount) of afferent fibers stained when recording from a unit tuned to 17 kHz, as seen from its auditory threshold curve (upper left). Arrow indicates anterior.

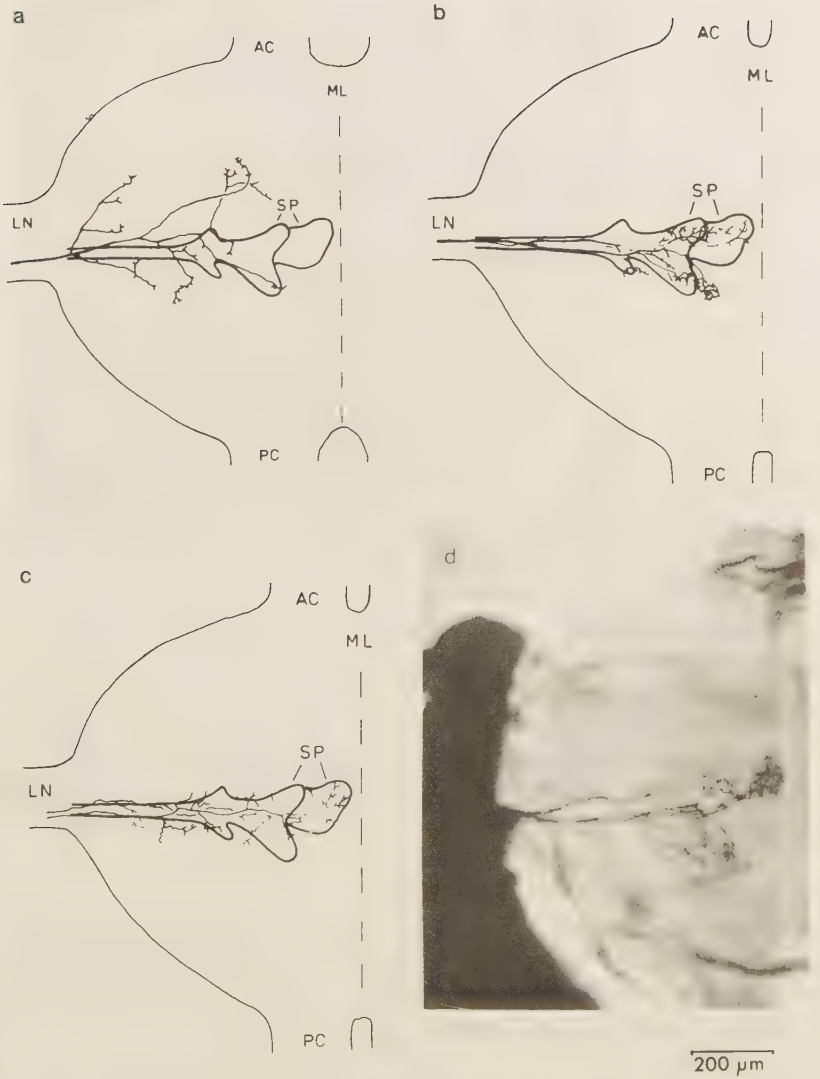
Fig. 24 Horizontal sketches of the crescent-shaped intermediate neuropile superimposed on the projection patterns of fibers revealed during individual recordings of the four physiologically separate receptor types. Arrow indicates anterior.

a. A sketch of Type 1 (2 kHz) projections

b. A sketch of Type 2 (4-5 kHz) projections

c. Projections of fibers stained while recording from a unit exhibiting sensitivity maxima at 4.5 and 11 kHz (Type 3).

d. Projections of fibers stained while recording from a unit best-tuned to 17 kHz (Type 4), taken from a wholemount.


a
AC
ML
SP
LN
PC
b
AC
ML
SP
LN
PC
c
AC
ML
SP
LN
PC
d
200 µm

fill does reveal 3 cell types also found in fills of the whole tympanal nerve; they probably arise from campaniform sensillae, the tympanal and/or the subgenual organ (Fig. 24d).

E. Processing of auditory information by a prothoracic interneuron

1. Morphological features

A large segmental auditory interneuron, the omega cell, which has been studied in several cricket species (Casaday and Hoy, 1977; Popov et al., 1978; Wiese, 1978; Wohlers and Huber, 1978a and b; Kleindienst et al., 1980) was investigated using intracellular recording and staining techniques. It is contained wholly within the prothoracic ganglion and was named the omega cell by Wiese (1978) on the basis of its shape (Fig. 25). The cell exhibits central arborizations which project to intermediate neuropile within both lateral hemispheres of the ganglion (Figs. 25 and 26), in a fashion similar to tnat of the auditory afferent fibers (see Fig. 18). Arborizations are also seen to course toward each lateral edge of the ganglion, apparently along the bundle of incoming auditory afferents (Popov et al., 1978; Wiese, personal communication). A relatively large axonal process (ca. 7 µm in diameter) crosses the midline within Ventral Commissure I (Gregory, 1974) and connects the two hemispherical portions. The neurite arises unilaterally from this axonal process and courses anteriorly and laterally to the cell body located near the dorso-lateral edge of the ganglion between prothoracic nerve 1 and the ipsilateral neck connective.

Typically, the omega cell shows a lateral asymmetry; the axonal process on the side opposite to the cell body bifurcates, the medial branch giving rise to the central arborization and the lateral branch to peripheral endings (Fig. 27a, the cell shown in Figs. 25 and 26 does not exhibit this division).

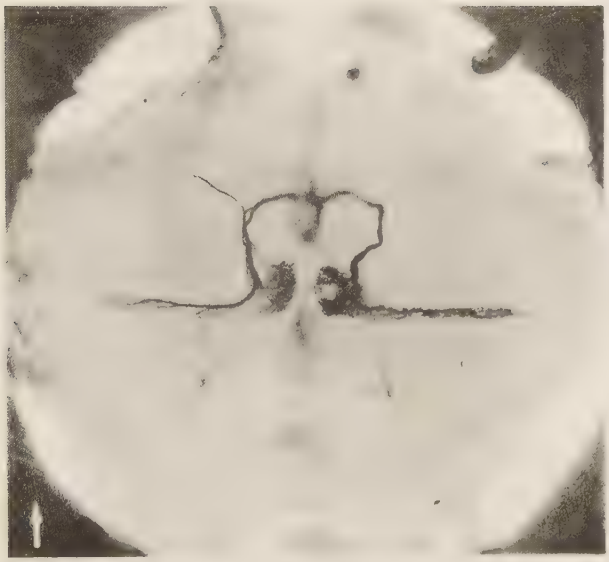


Fig. 25 Horizontal view of an omega cell, stained intracellularly. The cell body is out of the plane of focus (upper left). Arrow indicates anterior.

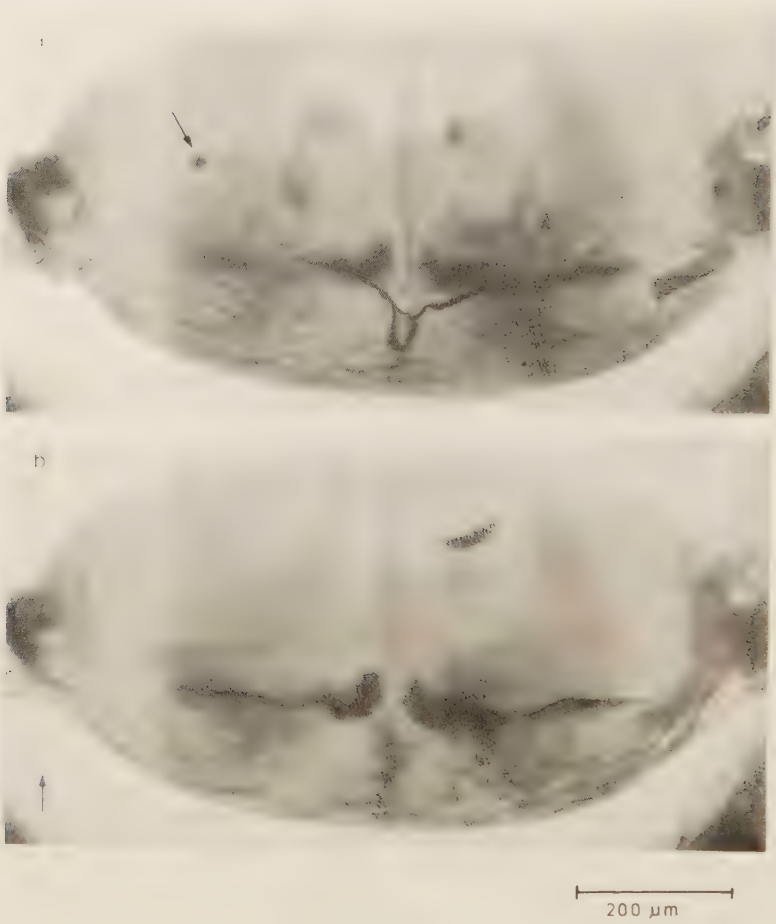


Fig. 26 Transverse optical sections of the omega cell shown in Fig. 25. Arrow indicates dorsal.

a. The most anterior part of an omega cell, the dorsolateral cell body (arrow) and the axon crossing the midline.

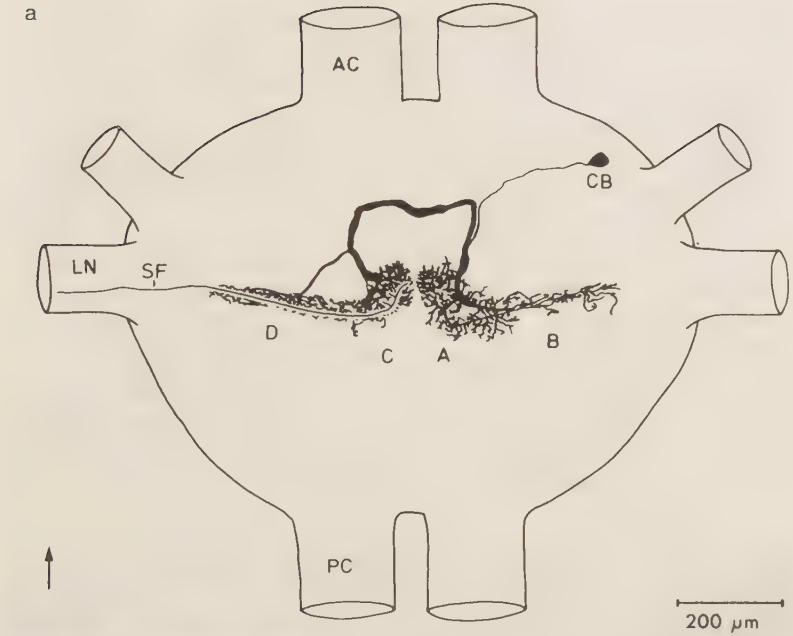
b. The arborizations within intermediate neuropile.

Fig. 27 The omega cell and its anatomical relationship to afferent fibers found within intermediate neuropile. Arrow indicates anterior.

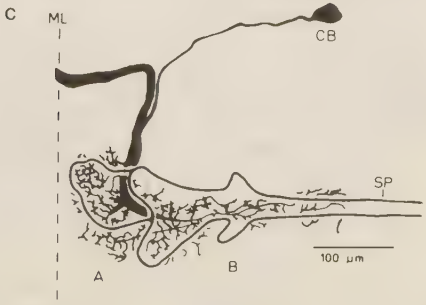
a. Reconstruction of an omega cell from horizontal sections. A single sensory fiber (SF) can be seen (left) coursing, along the lateral projections, toward the midline where it arborizes medially in the same area as the medial arborization of the omega cell. A,B,C,D, refer to the various fields of omega-cell arborization, based strictly upon their anatomy. AC, anterior connective; CB, cell body; LN, leg nerve; PC, posterior connective.

b. A photograph showing the left side of an actual section used in the reconstructions of the omega cell and sensory fiber seen in (a).

c. Horizontal reconstruction of the right side of the omega cell (shown in a) with superimposed crescent-shaped sensory projections (SP) of intermediate neuropile to demonstrate areas of overlap. A,B, refer to fields of omega-cell arborization. CB, cell body; ML, midline of the ganglion.


a
AC
CB
LN
SF
D
C
A
B
PC
200 µm

b


c
ML
CB
SP
A
B
100 µm

On morphological grounds, the arborizations are classified as four separate fields. Field A is the central field on the cell-body side and is the major input region from auditory afferent fibers. Field B is defined as the lateral processes on the cell-body side. Field C is the central region on the contralateral side, and field D refers to the peripheral endings arising from the lateral branch of the contralateral axon (Fig. 27a).

Occasionally a single afferent fiber stained along with an omega cell and was found to course from the posterior portion of the leg nerve, along the lateral projections of the omega cell, branching within the central area of omega-cell arborization (Fig. 27a and b). The cobalt was taken up by a process in close association with the area of the omega cell penetrated by the electrode.

Figure 27c shows the omega cell and superimposed crescent-shaped neuropile from subgenual and tympanal afferent fibers (see also Fig. 15), demonstrating areas of overlap. In one case when an omega cell was penetrated intracellularly in the region of central arborization, a large quantity of cobalt leaked into extracellular space and was taken up by many afferent fibers (Fig. 28), revealing both the omega cell (a) and the ball-shaped neuropile (b, also shown in Fig. 15) assumed to be true auditory neuropile.

2. Physiological features

The omega cell is an auditory interneuron which is most sensitive to the carrier frequency (4-5 kHz) of the calling song (Fig. 29). Figure 30 shows omega-cell responses to tones and patterned acoustic stimuli. The response to long tones of medium intensity and short rise and fall times is characterized by a depolarization which gives rise to sustained tonic activity reflecting stimulus duration (Fig. 30a). A post-stimulus depression (arrow) is observed in the membrane potential after cessation of the sound

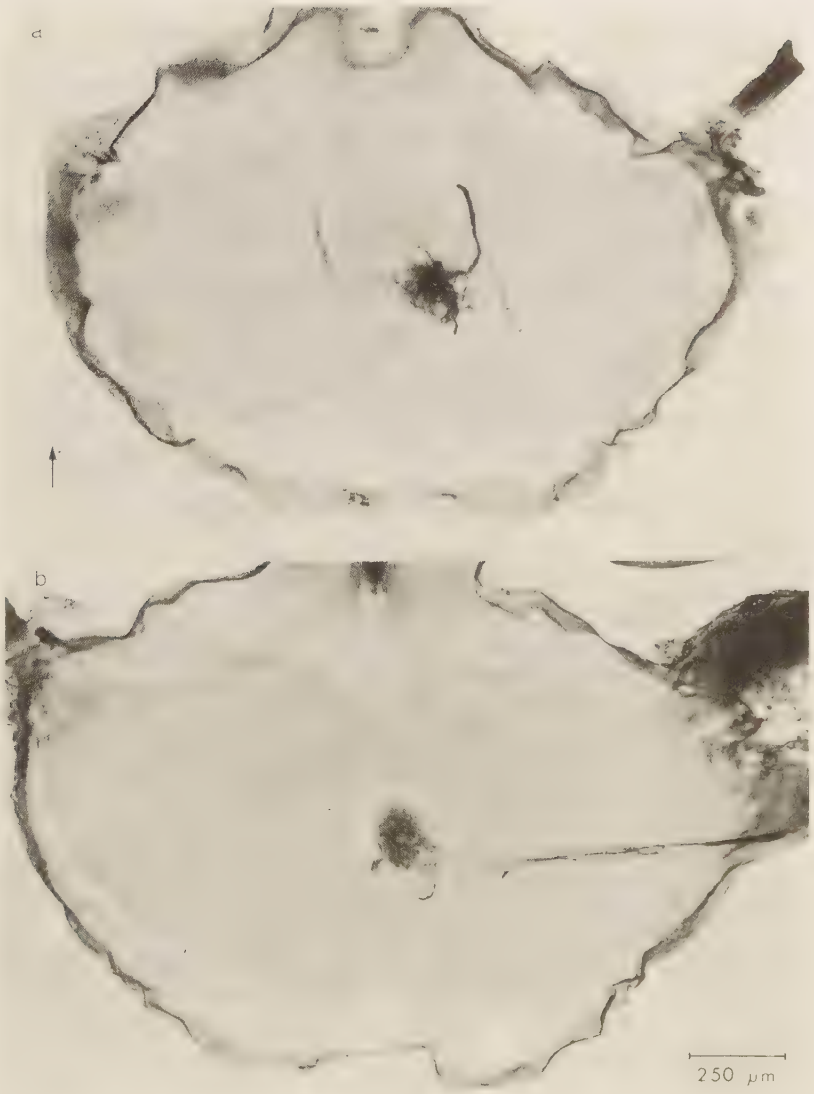


Fig. 28 Two consecutive horizontal sections (40 µm thick) taken from a stain of an omega cell (a) where a large quantity of cobalt leaked from the intracellular electrode into extracellular space and was taken up by a number of afferent fibers (b). Arrow indicates anterior.

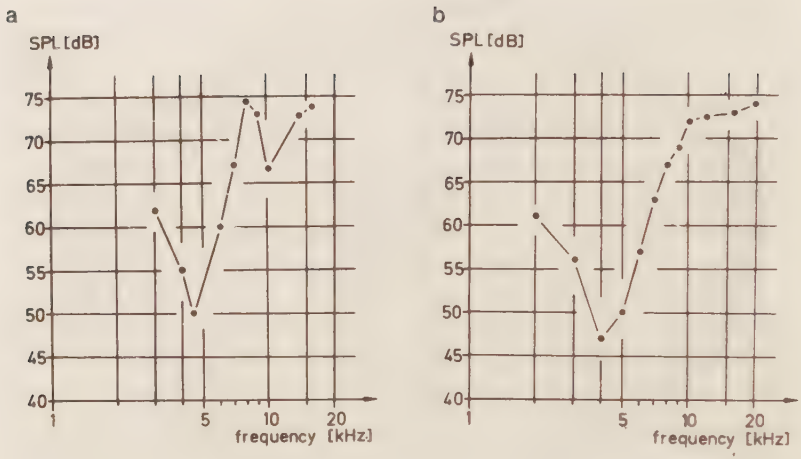


Fig. 29 Auditory threshold curves of the omega cell taken from two separate animals under open-field (a) and closed-field (b) stimulus conditions. (Modified from Kleindienst et al., 1980)

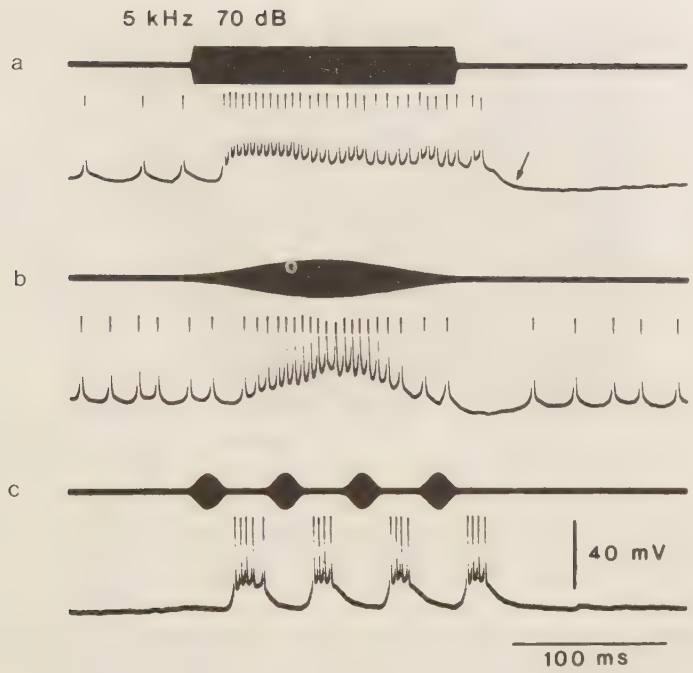


Fig. 30 Intracellularly recorded omega-cell responses to 140 ms tones with rectangular (a) and sinusoidal (b) envelopes, and to an artificial calling song (c). The arrow in (a) indicates a post-stimulus depression. Action potentials prior to the onset of the acoustic stimulus in (a) and (b) are due to a slight depolarization of the cell. (Modified from Wohlers and Huber, 1978)

signal. The stimulus envelope is reflected by both subthreshold activity and spike frequency (Fig. 30a and b). Temporal parameters of the calling song are also reflected accurately (Fig. 30c), with the first syllable eliciting a slightly stronger response than the fourth one. Syllable repetition rates both within the normal range for G. campestris (Weber, 1978) and those well outside this range are copied well, as shown in the computer-averaged data in Figure 31. Each syllable within a chirp elicits a sharply-timed response, and pauses are represented by a period of inactivity with respect to action potentials.

Intracellular records provide evidence for separation of input and output regions in the omega cell. Consecutive intracellular recordings (in different animals) were made from larger processes of central arborization in both lateral hemispheres and from the axonal process leading to the peripheral arborizations on the side contralateral to the cell body (Fig. 32). Electrodes penetrating processes in the central region ipsilateral to the cell body (Field A) register both synaptic and action potentials (lower right), indicating the proximity of afferent input. Intracellular electrodes on the side contralateral to the cell body only record action potentials (left); no synaptic potentials have ever been observed.

Crickets have two omega cells, as mirror-images of one another. The areas of arborization in each cell extensively overlap those of the mirror-image partner both medially and laterally (Fig. 33; also Wiese, 1978 and personal communication). For example, field A of one cell (input region) overlaps field C of the mirror-image cell. This configuration allows each cell to receive unilateral auditory information from the auditory afferents ipsilateral to the cell-body side.

Acoustic stimulation at above-threshold intensities under open-field conditions described earlier results in the

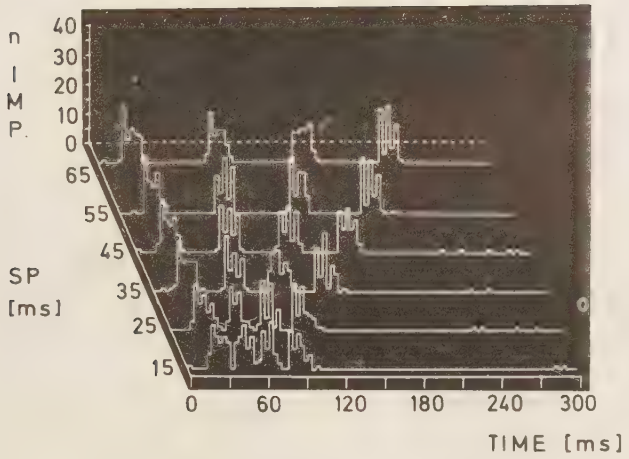


Fig. 31 Three-dimensional graph of the averaged response of an omega cell to artificial calling songs (4.5 kHz, 75 dB) using various syllable periods (SP). Normal syllable periods for G. campestris are 20-38 ms. Abscissa: Time in ms; Numbers to the left: Syllable periods (SP) in ms; Ordinate: Number of impulses summed over 10 chirps in bin widths of 2 ms. (Modified from Wohlers and Huber, 1978)

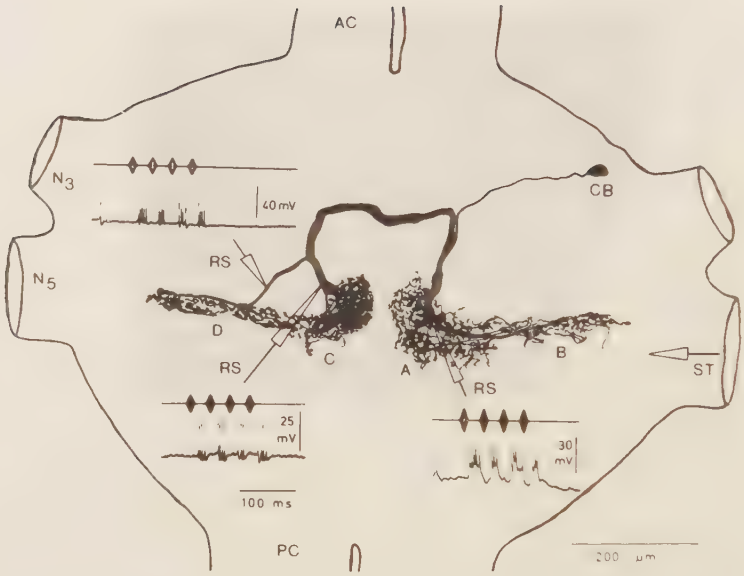


Fig. 32 Reconstruction of an omega cell, stained intracellularly. Labels: A,B,C,D, dendritic areas of the neuron; AC, anterior connective; CB, cell body; N3, nerve 3; N5, nerve 5 (leg nerve); RS, recording sites (shown with arrows); ST, side of stimulated ear. Insets: Right, intracellular recording showing synaptic activity (summed EPSPs) and superimposed spikes from field A (input area of the neuron) in response to an artificial calling song. Lower left, intracellular recording from field C and upper left, intracellular recording from the axonal branch to field D (probably output areas of the cell) with spikes correlated to each syllable of the chirp, but without noticable synaptic activity. Stimulus parameters were: 4.5 kHz, 75 dB. (Modified from Wohlers and Huber, 1978)

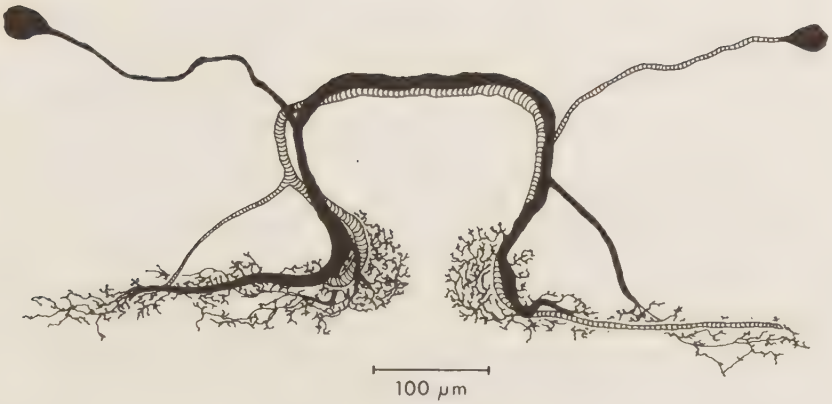


Fig. 33 Reconstruction (viewed from ventral) of two omega cells stained simultaneously in one animal, illustrating the close association they have to one another. One cell stained much more lightly than the other, thus allowing for separation of entangled processes. Fine details of the arborization are shown only for the cell drawn in solid black.

stimulation of both tympanal organs via the two tympana and simultaneously via the trachea and spiracular openings (Zhantiev et al., 1975a; Hill and Boyan, 1976 and 1977; Paton et al., 1977; Larsen and Michelsen, 1978; Kleindienst et al., 1980). Nevertheless, recordings from the omega cell, while stimulating from the ipsilateral (cell body) and contralateral sides successively, reveal differences in response characteristics (Fig. 34). Thresholds for ipsilateral stimulation at 5 kHz are consistently lower by up to 10 dB, the absolute threshold being 45-50 dB SPL. Increasing of both ipsilateral and contralateral stimulus intensity results in an enhanced response of the monitored omega cell, but at any response level the ipsilateral-contralateral intensity difference remains.

To further study the direction-dependent responses the closed-field stimulus system (legphones) was used to externally isolate each tympanum. The spiracles were always waxed over to create constant conditions and to eliminate them as an acoustic input to the tympanal organs. The stimulus system and some body structures associated with hearing are diagrammed in Figure 35. While recording intracellularly from an omega cell, the ipsilateral (excitatory input) and contralateral tympanal organs could be stimulated selectively. In addition, manipulations of the nervous system (for example severing of the contralateral leg nerve) and of the large trachea (see scissors) facilitated studies of the influence they have on the omega-cell response.

The frequency-threshold response under these closed-field conditions (Fig. 29b) is very similar to that shown for open-field stimulation (Fig. 29a) except that at 10 kHz the second sensitivity peak seen in open-field conditions disappears; the tuning to 4-5 kHz remains.

Stimulating the input side of an omega cell at 5 kHz while increasing the intensity yields the response curve plotted

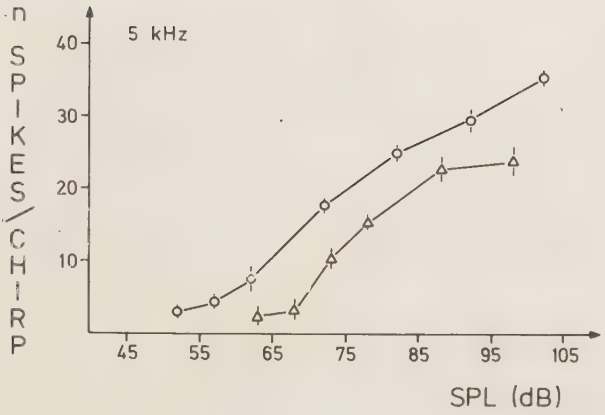


Fig. 34 Sound-intensity representation of an omega cell in response to an artificial calling song at 5 kHz for stimulation of the ipsilateral (circles) and contralateral (triangles) tympana under open-field conditions. The mean (from 20 chirps) and standard deviation are shown. (Modified from Wohlers and Huber, 1978)

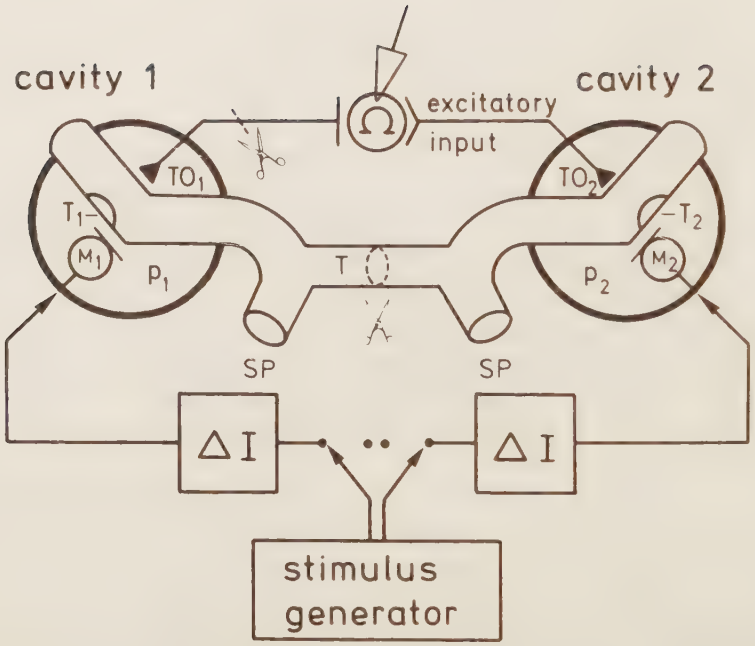


Fig. 35 Diagram of the closed-field stimulus system and tracheal structures associated with the hearing system to illustrate the stimulus conditions used in selective acoustic stimulation. Labels: Cavities 1 and 2, the sound cavities into which the legs were introduced to externally isolate them from one another; M1 and M2, Bruel and Kjaer 1/4 inch microphones (within the cavities) used as sound transmitters; P1 and P2, sound pressures produced in cavities 1 and 2 respectively; T1 and T2, the large tympana associated with cavities 1 and 2; TO1 and TO2, the tympanal organs associated with cavities 1 and 2; ΔI, sound attenuators to allow for independent manipulations of sound intensity reaching sound cavities 1 and 2; T, large trachea connecting the two tympana internally; SP, spiracles. The arrow (top) represents an electrode used to intracellularly monitor activity in an omega cell. (Modified from Kleindienst et al., 1980)

in Figure 36 (open circles). Extrapolation of the curve to the left results in a response threshold of about 48 dB SPL. An increase in sound intensity above this level produces a fairly linear increase in omega-cell response up to 65 dB SPL; the response saturates by 75 dB SPL. Stimulation of the contralateral tympanal organ yields the curve outlined by open triangles. This curve is shifted to the right; the threshold intensity is now above 80 dB SPL, and to elicit a response level of 22 spikes/stimulus, a sound pressure approximately 36 dB stronger must be delivered to the contralateral side relative to the ipsilateral stimulus producing an equivalent response (see arrows).

After cutting the contralateral leg nerve, thus elininating all contralateral auditory afferent input to the prothoracic ganglion, stimulation of the ipsilateral side results in a curve (closed circles) similar to the one produced with the nervous system intact. However, contralateral stimulation, which is effectively ipsilateral stimulation through the trachea since the contralateral tympanal organ can no longer excite cells in the ganglion, results in a shifting to the left (closed triangles) of the curve obtained from contralateral stimulation of the previously intact system (open triangles). Now the sound pressure needed on the contralateral side to produce 22 spikes/stimulus is only 11.5 dB more than that required from ipsilateral stimulation, indicating that activity of contralateral auditory afferents ultimately causes an inhibition of the omega cell when the nervous system is intact.

Such inhibition is seen in intracellular records. Figure 37 shows responses of an omega cell to ipsilateral and contralateral stimulation (closed-field) while recording from the area of field A (the large trachea was severed to eliminate the possibility of sound travelling through the trachea). Here ipsilateral stimulation excites the cell, triggering a spike response at above-threshold intensities (Fig. 37 a1 and b). Stimulation of the contralateral organ results in an active inhibition observed from

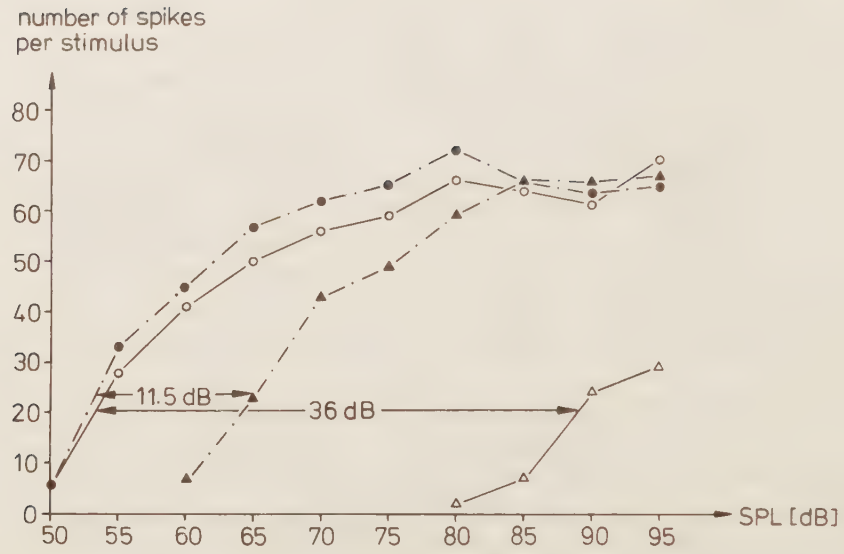


Fig. 36 Sound-intensity curves of an omega cell in response to ipsilateral and contralateral stimulation using the closed-field stimulus system diagrammed in Fig. 35. Abscissa: Stimulus intensity in dB SPL. Ordinate: Number of spikes per stimulus. Here a stimulus consists of six consecutive syllables 20 ms in duration and rise-fall times of 2 ms, presented at a rate of 5/sec and a sound frequency of 5 kHz. The open circles denote the curve obtained from stimulation of the input side (cell-body side) of the recorded omega cell. The open triangles refer to the curve obtained when stimulating the contralateral side. The closed circles outline the curve resulting from ipsilateral (input side) stimulation after severing of the contralateral leg nerve. The closed triangles denote the curve obtained from stimulation of the contralateral side with the contralateral leg nerve severed. (After Kleindienst et al., 1980)

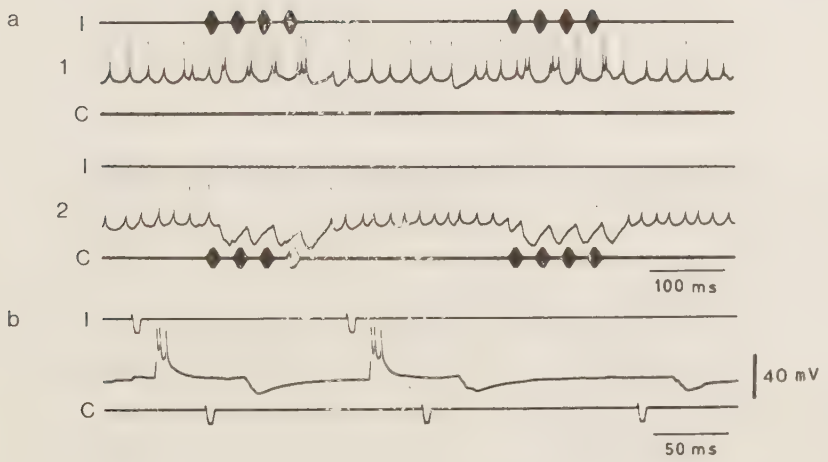


Fig. 37 Intracellular recordings from field A of an omega cell, using the closed-field stimulus system. In each series (a1, a2, and b) the upper trace (I) is the stimulus channel on the side ipsilateral to the cell body. The lower trace (C) is the stimulus channel on the contralateral side. The middle trace is the nervous activity recorded from field A. Stimulus parameters: (a) artificial calling song at 10 kHz, 65 dB. (b) stimulation with short 6 ms sound pulses at 12 kHz, 65 dB. (Modified from Wohlers and Huber, 1978)

field A (Fig. 37 a2 and b). The IPSP, as well as the EPSP activity is seen only from the area of field A and not from the contralateral side, thus establishing the cell-body side as both the excitatory and inhibitory acoustic input area (field A and/or B).

Spikes which are elicited in an omega cell as the result of excitation from auditory afferent fibers on the cell-body side travel, in a one-to-one relationship, from the spike-generating region near field A across the "bow" to the contralateral side, a phenomenon observed when simultaneously recording from fields A and C of a single omega cell (Fig. 38a). This relationship also holds true for spikes generated electrically through depolarization of field A using an intracellular electrode (Fig. 38b). The travel time for a spike from the cell-body side to the area of field C is approximately 0.2 ms, but it could not be shown clearly. With the large trachea severed, the excitatory-inhibitory effects can be studied relative to simultaneous stimulation of both tympanal organs when the nervous system is intact. Figure 39 shows the results of such an experiment. An intracellular electrode penetrated the area of field C and an extracellular electrode monitored spike activity from the input side (field A). Stimulation of the input side at 5 kHz and 70 dB SPL results in spike activity observed through both electrodes, a total of 8 spikes being elicited (Fig. 39a). When the same stimulus is presented to both the ipsilateral and contralateral tympanal organs simultaneously (Fig. 39b), the spike number is reduced to 50% (4 spikes) of the response elicited from only ipsilateral stimulation. This reduction is due to the inhibition from the contralateral side. The latency-to-first-spike does not appear to change significantly. Likewise, the response duration is approximately the same. The spike frequency however, is greatly reduced.

In a more quantitative way the excitatory-inhibitory effects are further shown. The ipsilateral tympanal organ was

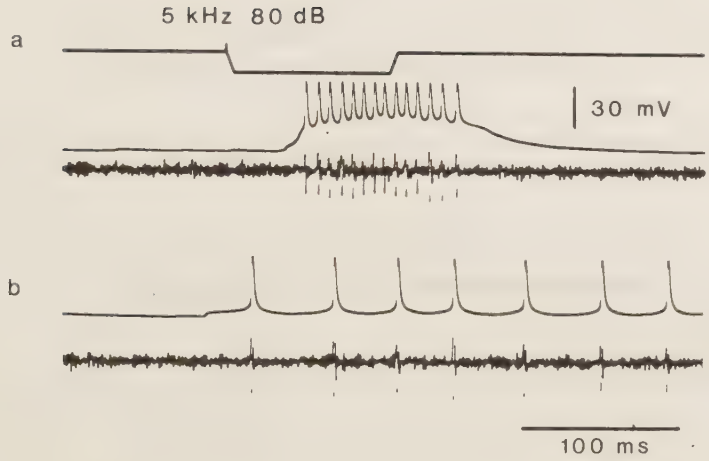


Fig. 38 Responses of an omega cell recorded intracellularly from field A (upper response trace in a and b) and extracellularly from field C (lower response trace in a and b).

a. Omega cell response to a sound pulse presented at 5 kHz, 80 dB (top trace).

b. Spikes produced by depolarizing the cell through the intracellular electrode on the input side (field A).

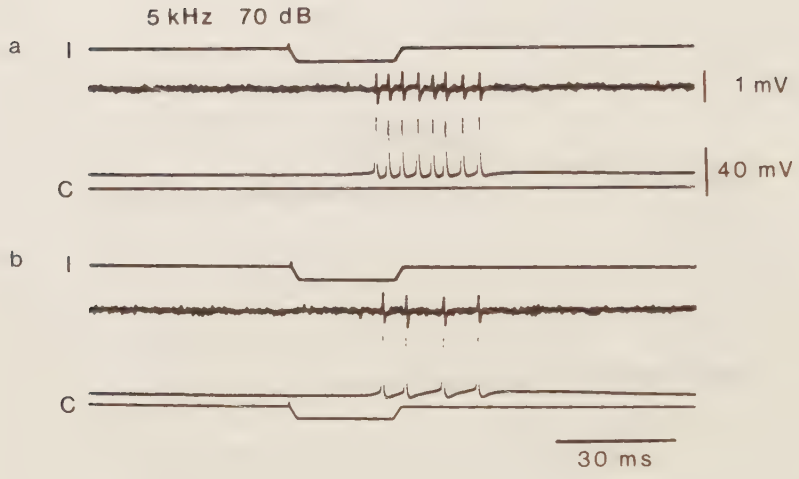


Fig. 39 The effect of contralateral inhibition on the spike response of an omega cell. In both (a) and (b) the upper trace shows the stimulus presented to the ipsilateral (I) side. The second trace is omega-cell activity recorded extracellularly from field A. Trace 3 is omega-cell activity recorded intracellularly from field C. The lower trace shows the occurrence of a stimulus presented to the contralateral (C) side. Stimuli were presented with the closed-field stimulus system; the large trachea was severed in the middle.

a. Response to a stimulus (5 kHz, 70 dB) presented only to the ipsilateral (input) side.

b. Response to the same stimulus presented simultaneously to both tympana.

stimulated at increasing sound pressures and the response was plotted against intensity (Fig. 40, closed circles). When an intensity eliciting a response of 62 spikes/stimulus was reached (65 dB, arrow), this level was held and the stimulus intensity to the contralateral side was increased in 5 dB steps from threshold to 95 dB SPL, yielding the curve plotted with open circles. The response in the omega cell is seen to decrease with an increase of intensity to the contralateral tympanal organ until at an intensity of 65 dB SPL on both sides the response is reduced to 43 spikes/stimulus. As the contralateral intensity increases even further, the response diminishes even more until at 90 dB a minimum response of 15 spikes/stimulus is reached. A further increase of sound pressure to the contralateral side results in a slight increase in the response, probably indicating a breakdown in the response pathway from the contralateral tympanal organ due to overstimulation.

Anatomical studies of the relationships the two omega cells have to one another led to the question of whether the mirror-image omega cell could be a part of the pathway eliciting an inhibitory response (from contralateral stimulation) of a given omega cell. To test that possibility, responses of both omega cells were simultaneously monitored, one intracellularly from the input side, and the other extracellularly from the opposite ganglionic hemisphere, as illustrated in Figure 41. Closed-field stimulus conditions were used and the large trachea was severed.

Under these conditions, a pulse of sound (5 kHz, 70 dB) delivered to the input side of the intracellularly-monitored omega cell triggers EPSPs and 10 spikes in that cell (Fig. 42a). The same sound pulse presented to the contralateral side results in a slight hyperpolarization of the intracellularly-recorded omega cell (arrow) and a simultaneous spike response (10 spikes) in the extracellularly-recorded omega cell (Fig. 42b). When the sound pulse is

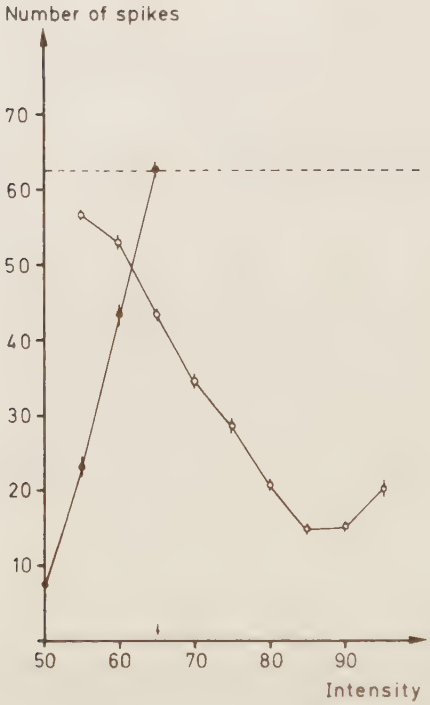


Fig. 40 Inhibition effects in the omega cell in response to increasing intensities of stimulus to the contralateral side, after severing the large trachea. The closed circles represent the sound-intensity curve obtained when stimulating only the input side. Open circles denote the curve obtained from stimulation of the input side at a constant intensity of 65 dB (arrow) while slowly increasing the intensity of the same stimulus simultaneously presented to the contralateral (inhibitory) side. Abscissa: Stimulus intensity in dB SPL. Ordinate: Average number of spikes per stimulus train. Here a train consisted of six pulses 50 ms in duration, rise-fall times of 2 ms, presented at a rate of 5/sec. The data points are averages of 5 such trains and also show the standard error. All pulses were presented at a sound frequency of 5 kHz.

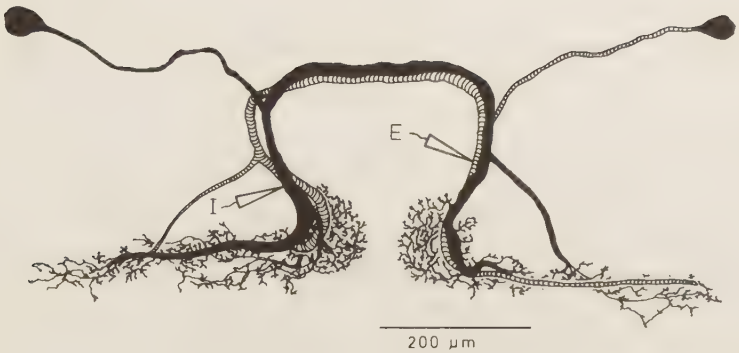


Fig. 41 Diagram of the recording situation used for collecting the data shown in Fig. 42. An intracellular electrode (I) penetrated field A of one omega cell and an extracellular electrode (E) recorded activity of the mirror-image cell in the opposite ganglionic hemisphere.

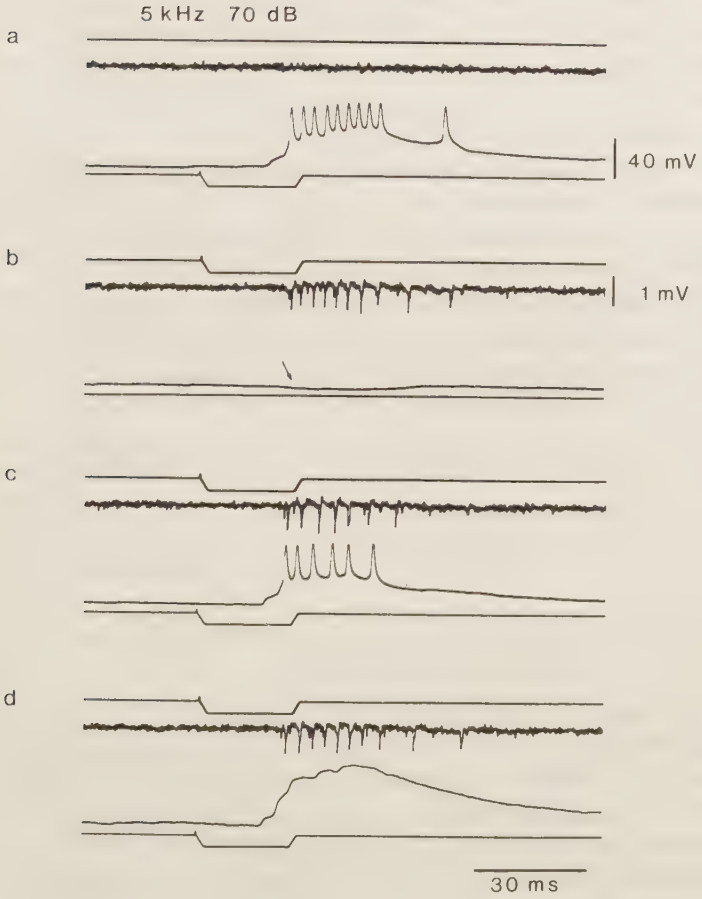
Fig. 42 The inhibitory effect of the mirror-image omega cells upon one another. In each series the upper trace is the stimulus channel connected to the input side of the extracellularly-recorded omega cell, the response of which is seen on the second trace. The lower trace is the stimulus channel connected to the input side of the intracellularly-recorded omega cell, the response of which is seen on the third trace. The large trachea was severed in the middle to eliminate its influence on bilateral stimulation. The stimulus had the same temporal parameters in every case and was always presented at 5 kHz, 70 dB SPL.

a. Response of the intracellularly-recorded omega cell to stimulation of the input side only. No response is seen in the extracellularly-recorded cell.

b. Response of the extracellularly-recorded omega cell to stimulation of its input side only. A slight hyperpolarization is seen in the intracellularly-recorded cell (arrow).

c. The response of both cells to simultaneous stimulation of both tympana. The spike response of both cells is lower than that seen in each cell when stimulated only from its input side (a and b).

d. The spike response of the extracellularly-recorded omega cell (second trace) to simultaneous stimulation of both tympana after the intracellularly-recorded omega cell was prohibited from spiking by hyperpolarizing the membrane electrically through the electrode (third trace, only an enormous summed EPSP can be seen). The spike response of the extracellularly-recorded omega cell (trace 2) is similar to the response shown in (b) which was obtained when only stimulating the input side of that cell.


5 kHz 70 dB
a
40 mV
b
1 mV
c
d
30 ms

simultaneously presented to both sides, a response is observed in both omega cells (Fig. 24c), the spike number in each cell being smaller than that produced when the input side of each omega cell is stimulated separately. After hyperpolarizing the intracellularly-recorded omega cell until the sound pulse no longer produces spike activity in that cell, the same simultaneous stimulation of the two sides results in a return of the response in the extracellularly-recorded omega cell to 10 spikes/stimulus (Fig. 42d), the same number produced from stimulation of just the input side; only an enormous EPSP can be observed in the now-hyperpolarized mirror-image cell. Therefore a given omega cell inhibits its mirror-image partner reciprocally.

IV. DISCUSSION

A. Elements in the tympanal nerve

Peripheral studies on the innervation of elements in the cricket tympanal nerve (Eibl, 1976 and 1978) revealed fibers arising from four different sensory structures: the tympanal organ, subgenual organ, tibial sensory hairs, and a contribution from tibia campaniform sensillae. From central stains of these fibers, a classification into four types can also be made on the basis of arborization within the prothoracic ganglion. The bundle of fibers contributing to ventral neuropile (Fig. 13) are known to arise from sensory hairs, a phenomenon common to several insect types (Honegger, 1977; Hustert, 1978; Tyrer et al., 1979; Wohlers et al., 1979). Afferent fibers from the campaniform sensillae project to areas of intermediate and dorsal neuropile. A similar result has been observed by Pflüger (personal communication) in the locust. Projections of subgenual and tympanal afferent fibers are anatomically

separate from these others; they form the crescent-shaped neuropile (Rehbein, 1973; Eibl, 1976; Eibl and Huber, 1979) consisting of the two fiber types reported in this study (see Fig. 15). A similar sensory neuropilar structure has been described by Kalmring et al. (1978) for the tettigoniid Decticus verruciverus L.

It could not be established, by cobalt-filling from the periphery, whether or not the two fiber types represent a separation of the two sensory structures contributing to this crescent-shaped neuropile. Two distinct bundles are observed; the most medially-projecting bundle must contain a large number of auditory afferent fibers because it widely overlaps areas of central arborization from auditory interneurons. Since the two sensory structures, the subgenual and tympanal organs, are so intimately related peripherally, degeneration studies similar to those performed on cockroaches by Boeckh et al. (1970) may prove valuable for separating fibers from the two sensory modalities according to their central "Gestalt".

The degeneration of axons within the tympanal nerve has just recently been studied by Michel (1979). After destroying the tympanal organ, she found that the largest axons within the tympanal nerve did not degenerate and assumed therefore, that they belong to receptors from the subgenual organ. Our finding of two types of central projections from subgenual and tympanal afferent fibers indicates either a separation of tympanal and subgenual afferents or, in the event that tympanal afferents contribute to both fiber types, a tonotopic organization or anatomical spacing of auditory neuropile. The separation of complex neuropiles into distinct modalities is also a problem in tettigoniids.

B. Characterization of single auditory receptors

Frequency discrimination in the cricket auditory system

begins at the level of the ear (Michelsen and Nocke, 1974; Hill and Boyan, 1977; Paton et al., 1977; Larsen and Michelsen, 1978). Recordings of summed activity from the tympanal nerve show two regions of enhanced sensitivity to auditory stimulation, one at the carrier frequency of the calling and rivalry songs (4-5 kHz in G. campestris) and another one correlated to higher frequencies found in the courtship song (Nocke, 1972; Zhantiev and Tshukanov, 1972a; Popov, 1973).

From single-cell recordings, four different cell types, based on frequency responses and, in part, on central structure, are seen. Those responding best to 2 kHz signals are physiologically characteristic of subgenual receptors which normally respond to substrate vibrations, but which also are capable of responding to low frequency airborne sounds up to 3 kHz (Dambach, 1972; Nocke, 1972; Dambach and Huber, 1974; Kalmring et al., 1978). But their central projections are similar to those of campaniform sensillae, though responses of campaniform sensillae to airborne sound have never been reported.

The units best-tuned to the carrier frequency of the G. campestris calling song (4-5 kHz) are thought to represent the majority of auditory afferents since they are most frequently encountered when recording from the auditory bundle within the leg nerve (Esch et al., 1980). Although single cells of this type can be studied physiologically, cobalt stains always reveal more than one fiber, all of which are contained within the crescent-shaped neuropile known to arise from subgenual and auditory receptors. Typically, one to several of the larger fibers contributing to lateral portions of the crescent shape are seen along with one or two thinner fibers projecting to the most medial portion of the crescent-shaped neuropile.

The auditory receptors exhibiting two sensitivity maxima, 4.5 and 11 kHz, are also seen to arborize within the

crescent-shaped neuropile, though stains consistently show more than one fiber. It can be argued that multiple-unit stains, combined with the physiological finding of two sensitivity maxima, strongly suggests that two or more fibers were recorded simultaneously. Additionally, the physiological records fail to show the existence of a single spike amplitude. If these really are multiple-unit recordings, then there must be a population of auditory afferent fibers which are best-tuned to 11 kHz, in addition to those tuned to 4-5 kHz. Otherwise this fiber type exhibiting two sensitivity maxima must exist.

Physiologically, receptors tuned to 17 kHz were also shown. In one case 3 fibers were stained, two of which represent both cell types found in the crescent-shaped neuropile. Here a single spike amplitude was observed throughout the experiment, thus supporting the existence of this cell type, even though it was only once encountered and could not be anatomically visualized.

Morphological characterization of the auditory receptor types is problematic because fibers are relatively small and tightly packed together in a bundle containing all the auditory receptors in addition to subgenual receptors, and probably, campaniform sensillae. Extracellular cobalt staining techniques (Rehbein et al., 1974) were used because of the small fiber diameters. But, when cobalt is introduced extracellularly, fibers surrounding the physiologically isolated one can also take up cobalt, making it impossible to determine which one yielded the physiological data. This problem is compounded when staining from such a bundle of tightly-packed auditory fibers as that found in the cricket. As recording techniques improve, it may become feasible to record intracellularly from some of the larger afferent fibers and thus selectively stain single units. Then the question of a possible central tonotopic organization of auditory receptors can be answered. Simultaneously, intracellular stains into the periphery

could be useful in determining whether the tympanal organ of the cricket has a tonotopic structure comparable to that suggested for tettigoniids (Zhantiev, 1971; Zhantiev and Korsunovskaya, 1977).

C. The omega cell

The omega cell is a segmental auditory interneuron found in the prothoracic ganglion of crickets (Casaday and Hoy, 1977; Popov et al., 1978; Wiese, 1978; Wohlers and Huber, 1978a and b) which joins the two lateral ganglionic hemispheres. Because of its relatively large size (ca. 7 µm in diameter) the cell could be studied using intracellular recording and staining techniques (Wohlers and Huber, 1978a and b). It could be positively identified from both its physiological response characteristics and its morphology such that morphological studies were not always necessary.

Spike responses of the cell to various auditory signals indicate that it is an auditory unit sending temporal information, received from afferents, to the next level of the auditory pathway without modifying the temporal structure. It is true that this cell responds best to the carrier frequency of the species-specific calling song (see Fig. 29). However, its frequency-response curve reflects that obtained from recordings of the whole tympanal nerve (Nocke, 1972), indicating that all of the auditory receptor types shown in this work converge on the cell, the majority of the receptors being tuned to 4-5 kHz. Nor does the pattern response of the omega cell appear to be any more sharply tuned than that of the afferent fibers.

The strongest evidence for a direct connection between auditory afferent fibers and the omega cell comes from anatomical studies. At the level of the light microscope, the arborizations of the omega cell are clearly seen to

overlap those of the most medially-projecting auditory afferent fibers. Latency differences between spike responses of auditory afferents and those of an omega cell indicate the possibility of a direct coupling (Wiese, personal communication). However, conclusive tests have never been performed.

Intracellular records give evidence for an anatomical separation of input and output regions. Postsynaptic potentials are observed exclusively from the cell-body side, and all spikes generated from that side travel across to invade fields C and D of the contralateral side.

Of special interest is the differential response to ipsilateral and contralateral stimulation (Fig. 37). Intensity differences at the level of the receptor cells are due to body shadowing (Kleindienst, 1978) and tracheal structures associated with the peripheral hearing system (Hill and Boyan, 1976; Paton et al., 1977; Larsen and Michelsen, 1978; Kleindienst et al., 1980). But the ipsilateral/contralateral response differences within the omega cell are shown in this study to be largely the result of a neuronal inhibition which disappears after the contralateral leg nerve is cut (Fig. 36). Furthermore, this inhibition comes largely, if not entirely, from the mirror-image cell (Fig. 42).

The precise connections have yet to be worked out; it may be that a functional connection between the two omega cells is made via another local interneuron. Independent of this uncertainty, however, the two omega cells, as mirror-images of one another, receive unilateral auditory input from the afferents ipsilateral to the cell-body side of each cell, and inhibit each other mutually. Such interaction could provide a neuronal basis for directionality which is expressed in the phonotactic behavior of the female, and must be further studied by looking closer at omega-cell outputs.

D. Other ascending auditory fibers

Auditory information is known to travel to the head ganglion (Stout and Huber, 1972; Popov et al., 1974; Rheinlaender et al., 1976; Hill and Boyan, 1977; Boyan, 1979; Stout and Huber, 1980). In G. campestris two types of ascending auditory interneurons have been reported, one reflecting the syllable and pause structure of the species-specific calling song (pulse-coder) and the other (chirp-coder) responding throughout the duration of a chirp without clearly coding the intersyllable pauses (Stout and Huber, 1972; Huber, 1977; Huber and Stout, 1978; Stout and Huber, 1980). Such a "chirp-coder" has also been reported by Popov et al. (1974) for G. bimaculatus. Furthermore, Wohlers and Huber (1978a and b) described an auditory interneuron with an ascending axon (AIAA) which is inhibited when stimulated at the carrier frequency of the calling song, but excited at frequencies above 10 kHz. How are these neurons related to the auditory afferents and/or the omega cell?

The pulse coding neuron appears to receive afferent information from those receptors best-tuned to the carrier frequency of the species-specific calling song, as reflected in its frequency tuning curve (Huber and Stout, 1978; Stout and Huber, 1980). Studies of a possible influence from an omega cell have not yet been initiated.

The chirp-coding interneuron responds to a broad frequency range (Stout and Huber, 1980) and probably receives afferent input from all auditory receptor types. Furthermore, it may be excited via both tympanal organs, thus increasing the duration of the response which could lead to a filling-up of the intersyllable pauses (Wohlers, unpublished results). Such a bilateral excitation could come directly from the auditory afferents of both tympanal organs, or in some way through interaction with an omega cell. The mechanism must be worked out from further anatomical and physiological studies.

A rather likely candidate for interaction with an omega cell is the AIAA. It is clearly inhibited in a frequency range where the omega cell is most sensitive. Its excitatory response to higher frequencies could be the result of massive input from auditory afferent fibers tuned to higher frequencies.

This fragmental anatomical and physiological picture of the auditory pathway in G. campestris indicates that the two important tasks for the female cricket, namely the identification of the conspecific song and localization of the sound source are laid down in the activity of a network of neurons. No conclusive neuronal model for sound recognition can be postulated from the present data; the overall picture of the detection process must still be drawn. This could be accomplished in part by simultaneously recording from several auditory neurons in order to study the relationships they have to one another. Results of such studies must be compared to those found from studies of phonotactic behavior in females (Weber et al., 1980).

V. SUMMARY

Crickets are well-known for their elaborate acoustical behavior used in intraspecific communication. The songs of males are perceived by tympanal organs of both conspecific males and females. This Thesis elucidates some aspects of the auditory pathway evolved for sound identification and sound localization.

1. Afferent fibers comprising the tympanal nerve of crickets arise from four different sensory structures, the tympanal organ, the subgenual organ, tibial campaniform sensillae, and some tibial sensory hairs. The fibers are classified according to their projection patterns within the prothoracic ganglion, being elucidated by cobalt staining methods. Sensory hair fibers contribute to ventral neuropile. Campaniform sensillae have relatively large axon diameters, and project to intermediate and dorsal regions of neuropile. Projections of fibers from the subgenual and tympanal organs exhibit a characteristic crescent shape within intermediate neuropile; they are distinctly different from the fiber projections of sensory hairs and campaniform sensillae. Though the fibers from the subgenual and tympanal organs can not be separated anatomically, two fiber types are seen within the crescent-shaped intermediate neuropile. The larger fibers are thought to outline neuropile belonging to subgenual receptors; the more medially-projecting thin fibers represent a major portion of the auditory neuropile.

2. Four types of afferent fibers could be identified by their physiological responses to acoustic stimulation and, in part, by their central projection pattern. Type 1 units respond best to signals of 2 kHz or less, and their arborizations are similar to those of campaniform sensillae. Type2 fibers are tuned to 4-5 kHz and are found to project to the crescent-shaped intermediate neuropile. Type 3 fibers have sensitivity maxima both at 4.5 and 11 kHz.

They also arborize within the crescent-shaped intermediate neuropile. A fourth type responds best to 17 kHz, but could not be characterized anatomically.

3. A segmental auditory interneuron, the omega cell, has arborizations within both lateral hemispheres of the prothoracic ganglion which overlap, and reflect, the crescent-shaped intermediate neuropile arising from tympanal and subgenual afferent fibers. This cell receives auditory input exclusively from the dendritic area ipsilateral to the cell body; the cell has a mirror-image partner. Both cells respond to sound signals from 2-20 kHz, being best-tuned to the carrier frequency of the cricket calling song (4-5 kHz for Gryllus campestris). A wide variety of temporal patterns are reflected in synaptic potentials and spike responses. The omega cell is excited by acoustic stimuli applied to the tympanal organ on the input side (the side ipsilateral to the cell body) and inhibited when stimulated via the contralateral tympanal organ. The major, if not entire, inhibitory input comes from the mirror-image omega cell. This interaction is probably the basis of a neuronal mechanism enhancing sound localization.

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